Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.
The interactive effects of nitrogen fertiliser and animal urine on nitrogen efficiency and losses in New Zealand dairy farming systems

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
Laura E. Buckthought

Lincoln University
2013
DECLARATION

This dissertation/thesis (please circle one) is submitted in partial fulfilment of the requirements for the Lincoln University
Degree of ________Ph.D.______

The regulations for the degree are set out in the Lincoln University Calendar and are elaborated in a practice manual known as House
Rules for the Study of Doctor of Philosophy or Masters Degrees at Lincoln University.

Supervisor’s Declaration

I confirm that, to the best of my knowledge:

- the research was carried out and the dissertation was prepared under my direct supervision;
- except where otherwise approved by the Academic Administration Committee of Lincoln University, the research was conducted in
  accordance with the degree regulations and house rules;
- the dissertation/thesis (please circle one) represents the original research work of the candidate;
- the contributions made to the research by me, by other members of the supervisory team, by other members of staff of the University
  and by others was consistent with normal supervisory practice.
- external contributions to the research (as defined in the House Rules) are acknowledged. (Delete if not applicable)

Supervisor __________________________ Date __________

Candidate’s Declaration

I confirm that:

- this dissertation/thesis (please circle one) represents my own work;
- the contribution of any supervisors and others to the research and to the dissertation/thesis (please circle one) was consistent with
  normal supervisory practice;
- external contributions to the research (as defined in the House Rules) are acknowledged. (Delete if not applicable)

Candidate __________________________ Date __________

Pre-Publication of Parts of this dissertation/thesis (please circle one)

Either:

1. We confirm that no part of this dissertation has been submitted for publication in advance of submission of the
dissertation/thesis (please circle one) for examination.

Candidate __________________________ Date __________
Supervisor __________________________ Date __________

2. Parts of this dissertation/thesis (please circle one) have been submitted and/or accepted for publication in advance of
submission of the dissertation/thesis (please circle one) for examination.

In this case, please set out on a separate page information on:

- which sections have been submitted, which have been accepted and which have appeared;
- which journals they have been submitted to;
- who are the co-authors.

Candidate __________________________ Date __________
Supervisor __________________________ Date __________
The interactive effects of nitrogen fertiliser and animal urine on nitrogen efficiency and losses in New Zealand dairy farming systems.

by

Laura E. Buckthought

The loss of nitrogen (N) through nitrate (NO$_3^-$) leaching and nitrous oxide (N$_2$O) emissions from pastoral dairy systems is one of the largest challenges facing the New Zealand agricultural industry. Nitrate leaching contributes to nutrient enrichment and accelerated eutrophication of streams, lakes and estuaries, while N$_2$O is both a greenhouse gas and the dominant anthropogenic emission contributing to stratospheric ozone depletion. Urine patches are the primary source of N loss from pastoral systems due to the high N loading that occurs over a relatively small area. Excessive or inappropriately timed fertiliser applications can also add to N loss.

Few studies have sought to determine the effect of concurrently deposited urine and fertiliser on the fate of N in pastoral systems, even though the application of fertiliser soon after grazing is commonly practised, while no studies have examined seasonal effects of any interaction. It is generally assumed that fertiliser applied over a urine patch will simply exacerbate the total N losses, as the urine-N saturates plant-N utilisation rates in pasture. This study, therefore, aimed to quantify the additional losses (if any) that occur as a result of fertiliser being applied concurrently onto a urine patch, and furthermore, determine the fate of the fertiliser N within the urine patch.

To determine this, a two year lysimeter study was undertaken where urine patches were applied in either autumn or spring. Urea fertiliser enriched with $^{15}$N was applied to these lysimeters at rates of either 200 or 400 kg N ha$^{-1}$ according to the standard regional practice. The amount of fertiliser derived N was measured in the leachate, N$_2$O emissions, pasture and soil. Fertiliser $^{15}$N recovered in leachate and N$_2$O emissions was $<$2.2% and $<$0.1%, respectively. Urine and fertiliser at the 400 kg N ha$^{-1}$ rate did increase total NO$_3^-$ leaching by up to 55 kg ha$^{-1}$ (p < 0.001), but this was as unlabelled N. Pasture uptake accounted for up to
52% of the fertiliser $^{15}$N recovery and increased plant uptake was observed under increasing fertiliser rates ($p < 0.001$). Recovery of fertiliser $^{15}$N in the soil at the end of the experiment was 22% with the majority of this in the top 10 cm soil. Total fertiliser $^{15}$N recovery ranged from 68-81% and it is suggested most of the unaccounted for $^{15}$N was lost as $N_2$ emissions and/or leached as dissolved organic N.

To further this work, data from the lysimeter experiment was validated against simulated results from a dynamic, process based model, APSIM (Agricultural Production Systems Simulator). The APSIM simulation was designed to mimic the experimental conditions and management of the lysimeter trial for the purpose of later using it to extrapolate the lysimeter data beyond its current confines to a larger range of treatment scenarios, climatic regions and soil types. Most of the modelled outputs were within the 95% confidence interval of the experimental data. However, the model significantly overestimated $N_2O$ emissions from under urine patches, and as a result, the existing simulation was not deemed suitable to immediately carry out extrapolative predictions from the lysimeter study. The key parameters potentially responsible for the overestimation of the modelled $N_2O$ emissions were identified, and a sensitivity analysis was performed on these parameters.

The lysimeter trial raised further questions as to how urine patches affect pasture N uptake and soil N dynamics in the field. Thus a field trial was established with the primary objective of determining the ‘effective area’ of a $^{15}$N labelled urine patch. Circular plots consisting of a urine patch ‘wetted area’ (0.28 m$^2$) and the potential ‘effective area’ were established and the pasture and soil N pools were monitored inside three distinct zones. A total of 22% of the urinary $^{15}$N was recovered in pasture outside the wetted area, mainly due to surrounding plant root proliferation. Recovery of urinary $^{15}$N in the soil was much smaller than in the pasture and was short-lived. Urinary N was recovered in the pasture up to 0.5 m from the edge of the wetted area, resulting in a total potential effective area of up to 2 m$^2$ (up to 6 times the wetted area).

In summary, this work has shown that when N fertiliser is applied over a urine patch at a rate of 400 kg N ha$^{-1}$ yr$^{-1}$ there is an increase in the amount of N leached, compared to urine alone, but that fertiliser N applied over a urine patch at 200 kg N ha$^{-1}$ yr$^{-1}$ does not increase the N loss. This study also suggests that improved validation and parameterisation of $N_2O$ emissions is required before the APSIM model can be used to produce accurate representations of the fate of N under urine and fertiliser deposition. Finally, using $^{15}$N recovery, the results suggest that the area affected by a urine patch is larger than the wetted area, primarily due to root extension and proliferation of the surrounding pasture.
Keywords: Nitrogen, nitrate leaching, nitrous oxide emissions, fertiliser, urine, pasture uptake, soil, APSIM, validation, effective area, wetted area, $^{15}$N recovery.
Acknowledgements

This project was carried out at AgResearch, located at the Ruakura Research Centre, Hamilton, New Zealand.

I would firstly like to acknowledge and thank my primary supervisor, Prof. Tim Clough. The perceived challenge of being located at a different campus was in fact no challenge at all due to his availability, and good communication. Any time I needed advice, motivation, or damage control, a phone call or teleconference sorted it out. Tim’s feedback was always thorough, constructive and remarkably fast.

Many thanks also to my co-supervisors, Mark Shepherd, for welcoming me to AgResearch and for the opportunity to undertake this project; Keith Cameron for his guidance and invaluable support with experimental design at the early stages; Hong Di for his timely feedback and for challenging my understanding of the modelled data; and Val Snow, for her help and patience while I learned the basic workings of APSIM, and for striking a valuable balance between pointing me in the right direction, and making me figure things out for myself.

For financial assistance I acknowledge and thank AgResearch, the Ingleby Company Ltd, MacMillan Brown and Alexanders Agribusiness Scholarships.

I am extremely grateful to the entire Nutrient Management and Environmental Footprinting team at AgResearch, Ruakura. Every single member of this team has helped me at some point. In particular, thank-you Amanda Judge for assisting me in organising the lysimeter collection and helping me settle in to life at Ruakura; and also to all those who assisted with field and lab work, including Stuart Lindsey, Jason McGrath, Sheree Balvert, Bill Carlson, Moira Dexter, Martin Kear, Paula Phillips, Brendon Welten, Alec McGowan, Mike Sprosen, Holly Sprosen, Justin Wyatt, Natalie Watkins, Bridget Wise, and Emma Bagley. At Lincoln University, I would like to thank Roger Cresswell for carrying out the $^{15}$N analyses, Manjula Premeratne for undertaking the N$_2$O analyses and Rob Sherlock for his advice and guidance.

I also sincerely thank John Waller for helping me immensely with the statistical analysis and his advice in general, and wish him a long and happy retirement. Also, Brian Atkins for turning my amateur drawing of a urine application guide into a lifesize stainless steel masterpiece.

To fellow students friends and colleagues at AgResarch, Lincoln and elsewhere thank-you for your support, in many different ways; Diana Selbie, Natalie Watkins, Jie Li, Paul Mudge,
Gina Lucci, Amy Van Wey-Lovatt, Debbie Clark, Dave Houlbrooke, Toni White, Kelly Rijswijk, Pranoy Pal, Nimlesh, Naomi Wells, Brendon Malcom and Fiona Curran-Cournane, thanks for your encouragement, funny jokes, and good company. Also, to my dear friends, Christina Painting, Michelle Budai, Courtney Murray, Elise Robinson, Gemma Greenshields, Leah Adlam, Amy Horrell, Claire Bradstreet, Elle Taylor, Robyn Blyth, Sandra Campbell and Vicky Underwood, thank-you for checking in, proof-reading, and support.

Finally to my family, Pip, Andrea, Mel, Evan, Marie, Pat and Joe and Michael and his family, thank-you all for your love, support, genuine interest and understanding.
# Table of Contents

Abstract ...................................................................................................................................... v  
Acknowledgements ................................................................................................................ viii  
Table of Contents ...................................................................................................................... x  
List of Tables ........................................................................................................................... xv  
List of Figures ........................................................................................................................ xvi  
Chapter 1 Introduction ............................................................................................................. 1  
Chapter 2 Literature review ....................................................................................................... 5  
2.1 Introduction  
2.2 The agricultural N cycle  
  2.2.1 The global importance of N in agriculture  
  2.2.2 Soil N in pastoral systems  
  2.2.3 Nitrogen inputs  
  2.2.4 Mineralisation and immobilisation  
  2.2.5 Nitrification  
  2.2.6 Plant uptake  
  2.2.7 Ammonia volatilisation  
  2.2.8 Leaching  
  2.2.8.1 Nitrate leaching  
  2.2.8.2 Dissolved organic N leaching  
  2.2.8.3 Measuring nitrate leaching  
  2.2.9 Denitrification and N₂O emissions  
  2.2.9.1 Measuring N₂O emissions  
2.3 Effects of the fate of N on the environment  
  2.3.1 Leaching  
  2.3.1.1 Mitigation/management of nitrate leaching  
  2.3.2 Nitrous oxide emissions  
  2.3.2.1 Mitigation/management of N₂O emissions  
2.4 Nitrogen return as urine  
  2.4.1 Urine composition  
  2.4.2 Area affected by urine patches  
  2.4.3 Fate of urinary N  
  2.4.3.1 Pasture  
  2.4.3.2 Microbial immobilisation  
  2.4.3.3 Leaching of urinary N  
  2.4.3.4 Gaseous emission of urinary N  
2.5 Nitrogen fertiliser in pastoral systems  
  2.5.1 Fate of fertiliser N  
  2.5.1.1 Pasture  
  2.5.1.2 Microbial immobilisation  
  2.5.1.3 Leaching  
  2.5.1.4 Gaseous N emissions  
2.6 Interaction of fertiliser N with urine patches  
  2.6.1 Precision agriculture in New Zealand  
2.7 Process based modelling in science  
  2.7.1 Types of models  
  2.7.2 Hierarchy and scale  
  2.7.3 Evaluation of models
2.7.3.1 Verification and validation 47
2.7.3.2 Sensitivity analysis 48
2.7.4 Limitations of modelling 48
2.8 Summary 50

Chapter 3 Urine patch and fertiliser N interaction: effects of fertiliser rate and urine timing on the fate of N ............................................................................................................ 52

3.1 Introduction 52
3.2 Methods and Materials
  3.2.1 Lysimeter Collection 53
  3.2.2 Bulk Density 56
  3.2.3 Experimental design and treatments 58
  3.2.4 Leachate collection and analysis
    3.2.4.1 Leachate $^{15}$N diffusion 61
  3.2.5 Nitrous oxide collection and analysis
    3.2.5.1 N$_2$O Sampling procedure 63
    3.2.5.2 Sample analysis 64
    3.2.5.3 Calculation of gas flux from lysimeters 64
  3.2.6 Pasture collection and analysis 65
    3.2.6.1 Pasture species composition 66
  3.2.7 Soil collection and analysis
    3.2.7.1 Gravimetric soil moisture 67
    3.2.7.2 Soil mineral N 67
    3.2.7.3 Soil microbial biomass N 67
    3.2.7.4 Soil total N and $^{15}$N 68
    3.2.7.5 Roots and stubble 69
  3.2.8 Isotope ratio mass spectrometry (IRMS) analysis 69
  3.2.9 Mass balance
    3.2.9.1 Fertiliser $^{15}$N recovery balance 70
    3.2.9.2 Apparent N balance 71
  3.2.10 Climate data 72
  3.2.11 Statistical analysis 72

3.3 Results
  3.3.1 Climate data 73
  3.3.2 Leachate
    3.3.2.1 Drainage 74
    3.3.2.2 Leached inorganic NH$_4^+$-N 75
    3.3.2.3 Leached inorganic NO$_3^-$-N 77
    3.3.2.4 Leachate inorganic $^{15}$N recovery 80
    3.3.2.5 Leached dissolved organic N (DON) 82
  3.3.3 Nitrous oxide
    3.3.3.1 Temporal N$_2$O 86
    3.3.3.2 Cumulative N$_2$O 91
    3.3.3.3 Temporal N$_2$O-$^{15}$N recovery 92
    3.3.3.4 Cumulative N$_2$O-$^{15}$N recovery 93
    3.3.3.5 Surface soil pH 94
  3.3.4 Pasture yield and N uptake
    3.3.4.1 Dry matter yield 95
    3.3.4.2 Pasture N content 98
    3.3.4.3 Pasture N uptake 99
    3.3.4.4 Pasture species composition 101
    3.3.4.5 Temporal fertiliser $^{15}$N recovery in pasture 102
    3.3.4.6 Cumulative fertiliser $^{15}$N recovery in pasture 104
Chapter 4 Validation of a process based simulation model, APSIM, with measured data from a lysimeter study

4.1 Introduction
4.2 Model validation methods
  4.2.1 Experimental approach
  4.2.2 Model description and settings
  4.2.3 Soils and climate
  4.2.4 Statistical analysis
4.3 Results
  4.3.1 Pasture growth and N uptake
  4.3.2 Water and solute movement through soil
    4.3.2.1 Drainage
    4.3.2.2 Leached NH$_4^+$-N
    4.3.2.3 Leached NO$_3^-$-N
  4.3.3 Ammonia Volatilisation
  4.3.4 Nitrous oxide emissions
  4.3.5 Total denitrification
  4.3.6 Denitrification vs nitrification derived nitrous oxide emissions
  4.3.7 Depth of nitrous oxide production
  4.3.8 Soil active carbon
  4.3.9 Soil inorganic N
  4.3.10 Soil water
4.4 Discussion
  4.4.1 Pasture growth and N uptake
  4.4.2 Water and solute movement through soil
    4.4.2.1 Drainage
    4.4.2.2 Leached NO$_3^-$-N
  4.4.3 Nitrous oxide emissions
  4.4.4 Soil inorganic N
  4.4.5 Validation discussion summary

Chapter 5 What is the ‘effective area’ of a urine patch?
List of Tables

Table 2.1: Atmospheric lifetimes and Global Warming Potential (GWP) values for common GHGs (IPCC, 2007). .............................................. 26
Table 2.2 Average nitrogenous constituents of cattle urine (Bristow et al., 1992). ............... 30
Table 2.3 Examples of frequency, volume, and surface area covered by dairy cattle urinations in grazed pasture systems ........................................ 31
Table 2.4 Hierarchical levels in plant science (Thornley and Johnson, 2000) ....................... 46
Table 3.1 Soil properties from lysimeter collection site ......................................................... 54
Table 3.2 Lysimeter treatments ............................................................................................. 58
Table 3.3 Lysimeter treatment application timing plan ......................................................... 59
Table 3.4 Occurrences of significant fertiliser treatment effects on N2O-N fluxes relative to the fertiliser application date ......................................................... 87
Table 3.5 Cumulative N2O-N fluxes for each treatment expressed as a % of total N applied, and as an emission factor ................................................................................. 92
Table 3.6 Soil bulk density at lysimeter collection site ............................................................. 108
Table 3.7 Fertiliser 15N recovered in soil (%) at each depth at the end of the trial ................. 114
Table 3.8 Mass balance of fertiliser 15N recovery from lysimeters at the end of the experiment (27 Aug 2012) ......................................................... 116
Table 3.9 Apparent Mass balance of treatment associated N recovery (urine and fertiliser) from lysimeters at the end of the experiment (27 Aug 2012) ................. 117
Table 4.1 Lysimeter experiment treatment descriptions ......................................................... 145
Table 4.2 Basic soil properties by horizon, used in the simulation (Close et al., 2003) ......... 149
Table 4.3 Soil hydraulic properties by horizon, used in the simulation (Vogeler, 2007) ....... 149
Table 4.4 Comparison of simulated cumulative denitrification, N2O and N2 emissions and measured N2O emissions from lysimeter experiment ..................................... 169
Table 5.1 Key soil properties (0-7.5 cm depth) at the field site in August 2011 ................. 200
Table 5.2 Treatments applied to plots .................................................................................... 201
List of Figures

Figure 2.1 The soil nitrogen cycle (Di and Cameron, 2002b). ................................................... 7
Figure 2.2 General pathway sequence of denitrification (Haynes and Sherlock, 1986). Values in brackets denote the oxidation state of N. .................................................. 18
Figure 2.3 Denitrifying enzyme activity at natural and modified soil pHs showing N₂ and N₂O production (Šimek et al., 2002). ................................................................. 19
Figure 2.4 Nitrification: pathways and enzymes involved (Wrage et al., 2001). ..................... 21
Figure 2.5 Nitrifier denitrification theoretical pathway (Wrage et al., 2001). ......................... 22
Figure 2.6 Global sources of N₂O production (Thomson et al., 2012). .................................. 27
Figure 2.7 Visible urine patches in the field. ........................................................................... 29
Figure 2.8 Distribution of bromide in the soil following simulated cattle urination events (Williams and Haynes, 1994). ................................................................. 33
Figure 2.9 Generalised response curve of pastoral herbage yield to N fertiliser application (Whitehead, 1995). ............................................................................. 38
Figure 2.10 Leached N from the Broadbalk Experiment at Rothamstead Experimental Station [Cameron et al. (2013) adapted from Goulding (2000)]. ................................. 39
Figure 2.11 Total NO₃⁻-N leached from lysimeters (a) without urine and (b) with urine. (DE is dairy effluent) (Silva et al., 2005). .............................................................. 41
Figure 2.12 Ten iterative steps for model development (Jakeman et al., 2006). ....................... 44
Figure 3.1 Lysimeter specifications (a) entire lysimeter; (b) the internal cutting ring; (c) lifting flanges; (d) lifting rods ................................................................................ 55
Figure 3.2 (a) to (g) Lysimeter collection process ..................................................................... 57
Figure 3.3 Individual lysimeter setup. ..................................................................................... 60
Figure 3.4 Leachate collection containers. ................................................................................ 61
Figure 3.5 Gas ring and headspace chamber on lysimeter...................................................... 63
Figure 3.6 Daily rainfall, mean air and soil (10 cm depth) temperatures and evapotranspiration at Ruakura over the duration of the experiment. ................................. 73
Figure 3.7 (a) Mean cumulative drainage and (b) mean cumulative drainage as a % of cumulative rainfall, from 21 February 2011 to 28 August 2012 for all treatments. Error bars = LSD (5%), n = 4. ............................................................................. 75
Figure 3.8 Mean NH₄⁺-N concentration vs (a) cumulative drainage and (b) time, from 28 Mar 2011 to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), n = 4. ............................................................................. 76
Figure 3.9 Cumulative NH₄⁺-N leached with (a) cumulative drainage and (b) time, from 28 Mar 2011 to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), n = 4. ............................................................................. 77
Figure 3.10 Mean NO₃⁻-N concentrations in leachate vs (a) drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. ............................................................................. 79
Figure 3.11 Cumulative NO₃⁻-N leached with (a) cumulative drainage and (b) time, from 28 Mar 2011 to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser
rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), n = 4.  

Figure 3.12  Mean cumulative fertiliser \(^{15}\)N recovery as NO\(_3\)-\(^{15}\)N in the leachate, from 21 Feb 2011 to 28 Aug 2012 for treatments that received \(^{15}\)N labelled fertiliser. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine, respectively. Bold arrows indicate urine + fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.  

Figure 3.13  Mean leached DON concentrations vs (a) cumulative drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.  

Figure 3.14  Mean cumulative leached DON concentrations vs (a) cumulative drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.  

Figure 3.15 Mean temporal N\(_2\)O emissions, from 21 Feb 2011 to 28 Feb 2012 from (a) nil urine, (b) autumn urine and (c) spring urine treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), error is only shown if LSR is > 5, n = 4. Note different y axis scales.  

Figure 3.16  Mean temporal N\(_2\)O emissions, daily rainfall and soil moisture, from 21 Feb 2011 to 28 Feb 2012 from (a) nil urine (b) autumn urine and (c) spring urine treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), error is only shown if LSR is > 5, n = 4. Note different y axis scales.  

Figure 3.17 Mean cumulative N\(_2\)O emissions, from 21 Feb 2011 to 28 Feb 2012 from all treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error = LSD (5%), n = 4.  

Figure 3.18 Mean recovery of N\(_2\)O-\(^{15}\)N from treatments that received \(^{15}\)N labelled fertiliser, from 21 Feb 2011 to 28 Feb 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + \(^{15}\)N fertiliser application, other arrows indicate split fertiliser \(^{15}\)N applications. Error bars = LSD (5%), n = 4.  

Figure 3.19 Mean cumulative recovery of N\(_2\)O-\(^{15}\)N from \(^{15}\)N affected treatments from 21 Feb 2011 to 28 Feb 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + \(^{15}\)N fertiliser
application, other arrows indicate split $^{15}$N fertiliser applications. Error bar = LSD (5%), n = 4. .................................................................94

Figure 3.20 Soil surface pH of lysimeters for all treatments, from 21 Jul 2011 to 19 Jan 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. ................................................................................95

Figure 3.21 (a) Temporal pasture DM yields and (b) cumulative pasture DM yields from all treatments, from 21 Feb 2011 to 28 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. ........................................................................................97

Figure 3.22 Pasture N content (%) from all treatments, from 21 Feb 2011 to 28 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. .......................................................................................99

Figure 3.23 (a) Temporal pasture N uptake and (b) cumulative pasture N uptake from all treatments from 21 Feb 2011 to 28 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. .......................................................................................101

Figure 3.24 Mean proportion of grass, weed and clover species in harvested pasture on 2 Jul 2012. Error bars = (5%), n = 4. .......................................................................................102

Figure 3.25 (a) Pasture atom% and (b) pasture $^{15}$N recovery from $^{15}$N labelled fertiliser treatments, from 21 Feb 2011 to 24 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4. .......................................................................................104

Figure 3.26 Mean cumulative pasture $^{15}$N recovery from all treatments that received $^{15}$N labelled fertiliser from 21 Feb 2011 to 24 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Arrows indicate N fertiliser application; bold arrows indicate urine + N fertiliser application. Error bar = LSD (5%), n = 4. .......................................................................................105

Figure 3.27 Mean dry matter content in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4. .................106

Figure 3.28 Mean N content (%) in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4. .................106

Figure 3.29 Mean N uptake in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4. .................107

Figure 3.30 Mean fertiliser $^{15}$N recovery in the roots and stubble at the end of the experiment for each treatment that received $^{15}$N labelled fertiliser. LSD (5%) is shown for (a) roots and (b) stubble, n = 4. .................108

Figure 3.31 Mean gravimetric soil moisture with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4. .......................................................................................109
Figure 3.32 Mean soil NH$_4$$^+$-N concentrations with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error = LSD (5%), n = 4. 110

Figure 3.33 Mean soil NO$_3$$^-$-N concentration with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4. 111

Figure 3.34 Total soil inorganic N comprising soil NH$_4$$^+$-N and NO$_3$$^-$-N at the end of the trial summed over the 0 to 70 cm depth. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. LSD (5%) is shown for (a) NH$_4$$^+$-N and (b) NO$_3$$^-$-N, n = 4. 111

Figure 3.35 Mean total soil N (%) with depth at the end of the trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4. 112

Figure 3.36 Mean total soil N (kg ha$^{-1}$) in lysimeters at the end of the trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4. 113

Figure 3.37 Mean soil microbial biomass N with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4. 113

Figure 3.38 Fertiliser $^{15}$N recovered in soil. Values represent the $^{15}$N recovery summed over all depths. 114

Figure 3.39 Root proliferation of barley in localised supplies of phosphate, nitrate, ammonium and potassium. Control (HHH) received a complete nutrient solution to the entire root system. Letters denote L, low; and H, high, referring to the nutrient concentrations available in the upper, middle and lower sections of the root mass, the middle section being between the two lines. Data from Drew (1975); adapted by Hodge (2004). 120

Figure 3.40 Relationship between the apparent recovery (%) of N in the pasture with the apparent recovery of inorganic N, DON and the sum of inorganic N+DON in the leachate from each lysimeter. 138

Figure 4.1 Components of the soil water and solute balance that are addressed by SWIM (Verburg et al., 1996a). 146

Figure 4.2 (a) Horotiu silt loam profile (Waikato Regional Council, 2011) and (b) soil horizon profile at lysimeter collection site. Both profiles are the same scale. 149

Figure 4.3 Daily rainfall, mean air and soil (10 cm depth) temperatures and evapotranspiration at Ruakura over the duration of the experiment. 151

Figure 4.4 Daily DM yield simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 20. 154

Figure 4.5 Cumulative pasture DM yield simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser

xix
applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. .................................................................155

Figure 4.6  Daily pasture N uptake simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 19. .................................................................156

Figure 4.7 Cumulative pasture N uptake simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. ......................................................................... 157

Figure 4.8  Daily drainage simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 21. ............................................................................... 159

Figure 4.9 Cumulative drainage simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. ........................................................................................ 160

Figure 4.10 Daily leached NO₃⁻-N flux simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over cumulative drainage. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 21. .................................................................................. 163

Figure 4.11 Cumulative leached NO₃⁻-N vs cumulative drainage simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments. Note: F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. Also note the Y axes are different. ..............................................................................................................164

Figure 4.12 Cumulative NH₃-N volatilisation over time for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹, respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. .......................................................................................................165

Figure 4.13 Daily N₂O emissions simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2

xx
and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 371.

Figure 4.14 Cumulative N\(_2\)O emissions simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. Also note the Y axes are different.

Figure 4.15 Cumulative denitrification simulated in APSIM for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + fertiliser application in either autumn or spring; other arrows indicate split fertiliser applications.

Figure 4.16 Total denitrification simulated in APSIM with relative proportions of N\(_2\)O and N\(_2\) products for all treatments.

Figure 4.17 Total N\(_2\)O production simulated by APSIM at incrementing depths from 0-700 mm. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively.

Figure 4.18 Mean soil active C simulated by APSIM at incrementing depths from 0-700 mm. F0, F2 and F4 denote treatment fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine treatment applications, respectively.

Figure 4.19 Soil urea simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\), respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note the Y axes differ.

Figure 4.20 Soil NH\(_4\)\(^+-\)N simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\), respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note Y axes differ.

Figure 4.21 Soil NO\(_3\)\(^-\)-N simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\), respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note Y axes differ.

Figure 4.22 Soil water simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\), respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. DUL is the water content at the drained upper limit; SAT is the saturation point; LL is the lower limit for plant uptake at 15 bars of pressure. Note the y axes differ...
Figure 4.23  Example of how the hydraulic properties of SAT, DUL, LL and oven dry soil are used to generate a continuous retention curve for (a) water content, and (b) hydraulic and saturated conductivity in a silt loam soil (Huth et al., 2012). ......189

Figure 5.1 Layout of the annular rings in an individual plot. .................................201
Figure 5.2 A single plot with the stainless steel template illustrating the segmented sampling approach. .................................................................203

Figure 5.3 Daily (left axis) and cumulative (right axis) rainfall at the field site, from 1 October 2011 to 1 August 2012. .................................................................211
Figure 5.4 Mean daily air and soil temperature (10 cm depth) at the field site, from 1 October 2011 to 1 August 2012. .................................................................212
Figure 5.5 (a) Urine patches visible prior to the first yield harvest and (b) pasture growth from a urine patch one week after the first harvest. .........................................213
Figure 5.6 Mean (weighted) pasture yields from the whole plot area, from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................213
Figure 5.7 Mean pasture yields, from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................215
Figure 5.8 Mean (weighted) cumulative pasture yields from the whole plot scale, from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................216
Figure 5.9 Mean cumulative pasture yields from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................217
Figure 5.10 Cumulative pasture yield (a) at the plot scale (weighted means) and (b) from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................218
Figure 5.11 Mean (weighted) pasture N content from whole plot area from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................219
Figure 5.12 Mean N content from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................220
Figure 5.13 Mean (weighted) pasture N uptake from whole plot area (mean of Zones A, B and C) from days 0 to 239. Error bars = LSD, n = 4.................................................................221
Figure 5.14 Mean pasture N uptake from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................223
Figure 5.15 Mean (weighted) cumulative N uptake from the whole plot area, from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................224
Figure 5.16 Mean cumulative pasture N uptake from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.................................................................225
Figure 5.17 Mean cumulative N uptake (a) from the plot scale and (b) from Zones A, B and C for all treatments. Error = LSD (5%), n = 4.................................................................226
Figure 5.18 Pasture $^{15}$N recovery over time from Zones A, B and C for treatments (a) urine + fertiliser and (b) urine only from day 0 to 310. Error = LSD (5%), n = 4.................................................................227
Figure 5.19 Cumulative pasture $^{15}$N recovery from Zones A, B and C for treatments (a) urine + fertiliser and (b) urine only, from day 0 to 310. Error = LSD (5%), n = 4.................................................................228
Figure 5.20 Mean total cumulative pasture $^{15}$N recovery (%) from Zones A, B and C for urine + fertiliser and urine only treatments. Error = LSD (5%), n = 4.................................................................229
Figure 5.21 Total cumulative pasture (a) $^{15}$N uptake and (b) background soil N uptake with distance from the centre of the urine patch in Zones A, B and C. Note the y axes differ.

Figure 5.22 Pasture $^{15}$N enrichment determined from cuttings up to day 42 (indicated by red arrow). From day 42 onwards values are from pasture harvests. Dashed line indicates enrichment of the applied urine (5 atom%). Error = LSD (5%), n = 4.

Figure 5.23 Soil moisture content at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4.

Figure 5.24 Soil NH$_4^+$N concentrations at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth under urine + fertiliser (U+F+), urine only (U+F0), fertiliser only (U0F+) and the control (U0F0) from day 0 to 106. Error bars = LSD (5%), n = 4. Note the y axes differ.

Figure 5.25 Soil NH$_4^+$N concentration at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ.

Figure 5.26 Soil NO$_3^-$N concentrations at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth under urine + fertiliser (U+F+), urine only (U+F0), fertiliser only (U0F+) and the control (U0F0) from day 0 to 106. Error bars = LSD (5%), n = 4. Note the y axes differ.

Figure 5.27 Soil NO$_3^-$N concentration at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ.

Figure 5.28 Soil N content (%) at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth for treatments (U+F+) urine + fertiliser; (U+F0) urine only; (U0F+) fertiliser only and (U0F0) control, from day 0 to 106. Error = LSD (5%), n = 4.

Figure 5.29 Soil N content (%) at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4.

Figure 5.30 Soil total N (kg N ha$^{-1}$) at the plot scale from 0-20 cm depth (sum of 0-7.5 and 7.5-20 cm depths) from (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control treatment from day 0 to 106. Error = LSD (5%), n = 4.

Figure 5.31 Soil total N (kg N ha$^{-1}$) from Zones A, B and C from 0-20 cm depth (sum of 0-7.5 and 7.5-20 cm depths) from (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control treatment from day 0 to 106. Error = LSD (5%), n = 4.

Figure 5.32 Soil inorganic $^{15}$N recovery (NH$_4^+$-$^{15}$N + NO$_3^-$-$^{15}$N) from day 0 to 43 from the urine + fertiliser treatment at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth and from the urine only treatment at (c) 0-7.5 cm depth and (d) 7.5-20 cm depth. Error bars = LSD (5%), n = 4.

Figure 5.33 Soil organic $^{15}$N recovery from day 0 to 43 from the urine + fertiliser treatment at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth and from the urine only treatment at (c) 0-7.5 cm depth and (d) 7.5-20 cm depth. Error bars = LSD (5%), n = 4. Organic $^{15}$N recovery calculated as the difference of total and inorganic $^{15}$N recovery.

Figure 5.34 Soil $^{15}$N recovered from the inorganic and organic fractions at the end of the trial at (a) 0-7.5 cm and (b) 7.5-20 cm depths. Error = LSD (5%), n = 4.
Figure 5.35 Soil total $^{15}$N recovery from day 0 to 106 from the urine + fertiliser treatment at (a) 0-7.5 cm and (b) 7.5-20 cm depths and from the urine only treatment at (c) 0-7.5 cm and (d) 7.5-20 cm depths. Error bars = LSD (5%), n = 4. ..............247

Figure 5.36 Soil microbial biomass N ($\mu$g g$^{-1}$ soil) at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth for treatments (U+F+) urine + fertiliser; (U+F0) urine only; (U0F+) fertiliser only and (U0F0) control from days 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ. ....................................................................................................................248

Figure 5.37 Soil microbial biomass N ($\mu$g N g$^{-1}$ soil) at 0-7.5 cm depth from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control, from day 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ. ....................................................................................................................249

Figure 5.38 Soil microbial biomass N ($\mu$g N g$^{-1}$ soil) at 7.5-20 cm depth from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control, from day 0 to 106. Error = LSD (5%), n = 4..................................................250

Figure 5.39 Soil microbial biomass $^{15}$N recovery (%) at the plot scale in the 0-7.5 cm depth, from the (a) urine + fertiliser and (b) urine only treatments, from day 0 to 43. Error = LSD (5%), n = 2. .................................................................251

Figure 5.40 Soil microbial biomass $^{15}$N recovery (%) at the 7.5-20 cm depth from Zones A and B for treatments (a) urine + fertiliser and (b) urine only, from day 0 to 43. Error = LSD (5%), n = 4. .................................................................................................................252

Figure 5.41 Visual pasture response to urine in Zones A, B and C of (a) urine treatment, and (b) non-urine treatment, one week after the first harvest (day 21). The red line indicates the perimeter of the visual pasture response to urine.................................255
Chapter 1
Introduction

The loss of nitrogen (N) from pastoral agriculture in New Zealand, particularly dairy systems, is a significant challenge faced by the New Zealand agricultural industry, and its mitigation has been at the forefront of much research over the past two decades and still is today. Losses of N represent not only a loss of soil fertility and potential productivity, but are also a considerable threat to the environment (Cameron et al., 2013). Nitrate (NO₃⁻) leaching can contribute to nutrient enrichment and the accelerated eutrophication of streams, lakes and estuaries, and agriculturally sourced nitrous oxide (N₂O) emissions contribute to climate change and the depletion of the ozone layer (Ravishankara et al., 2009; Sutton et al., 2011).

Of the N ingested by ruminant cattle, 60-90% is returned to the pasture in the form of urine with loading rates of individual urine patches reaching up to 1200 kg N ha⁻¹ (Haynes and Williams, 1993; Jarvis et al., 1995; Di and Cameron, 2002b). The pasture can only utilise a small proportion of this high N loading, leaving the remainder at risk of being leached (Cameron et al., 2013). This makes urine patches the most significant contributor to N leaching from agricultural soils (Haynes and Williams, 1993; Di and Cameron, 2002b) and many studies have characterised leaching losses from soils under different experimental conditions using lysimeters.

Although it is generally understood that split applied, well timed N fertiliser applications do not significantly contribute to NO₃⁻ leaching, it has been suggested that fertiliser application over urine affected areas increases the risk of N leaching and N₂O emissions and decreases fertiliser N use efficiency (Silva et al., 1999; de Klein et al., 2001; Mackenzie et al., 2011; Cameron et al., 2013). As a result of this, research has focussed on developing variable rate technologies for precision fertiliser application that are designed to selectively apply fertiliser to pasture while avoiding urine and dung patches (Yule and McVeagh, 2011).

However, few studies have actually quantitatively investigated the interaction between concurrent mineral N fertiliser application and urine patch deposition and its effect on N losses from pastoral systems. Furthermore, very few studies have differentiated the fertiliser-associated N losses from the urinary N losses. So if the environmental effects associated with applying fertiliser over urine patch areas have never been quantified, then what is the justification for the development of technology to avoid their co-application? There is therefore, a need to determine (a) the additional losses (if any) that arise from the application
of fertiliser over urine patches, and (b) the potential benefits (i.e. reduction in N loss) that can be achieved by urine patch avoidance technology, and whether, or not, it is a worthwhile strategy for N loss mitigation.

1. The first key objective of this study was therefore to understand the interaction of N fertiliser and urine on the fate of N, where fertiliser is applied following urine deposition, and determine to what extent, if any, N fertiliser enhances N losses from a urine patch.

A lysimeter methodology was chosen to answer this objective because lysimeters are a good experimental option for determining the fate of N from urine and fertiliser treated soil as they allow for the accurate measurement of water and solute drainage, pasture content and gaseous emissions from the soil surface. However, the extent of the treatment range and replication are limited by the high cost of lysimeter collection and installation. This is one area where agricultural models have proved to be highly valuable in science, as they can provide predictions and extrapolations where experimental measurement is constrained (Jakeman et al., 2006; Tedeschi, 2006).

The use of dynamic models to simulate pastoral systems in New Zealand and predict the transformations and fate of N is becoming increasingly relied upon to the point where modelled outputs are being interpreted by non-modellers and used as decision support mechanisms. This is not necessarily a bad thing, but it does increase the risk of models being used for purposes other than what they were originally intended for, potentially making the results meaningless in the context of their use (Jakeman et al., 2006). Furthermore, predictions from model outputs are also being used for regulatory purposes by councils and governmental organisations. It is therefore absolutely imperative that there is confidence in the performance of models and that users understand the limitations of the modelled outputs. Model validation using experimental data is a key step in model evaluation (Bennett et al., 2013), where a good degree of fit between modelled and experimental data is generally (but not exclusively) an indicator of a good representation of the system.

For this study, the Agricultural Production Systems Simulator (APSIM), a dynamic, process-based model was chosen as a suitable model through which to extrapolate and build on the experimental data obtained in answering Objective 1. APSIM was chosen because of its effective modular framework developed specifically to simulate biophysical processes in the whole farm system (Keating et al., 2003). APSIM’s management modules also allow the user to specify rules (e.g. pasture harvest dates and residuals) that characterise and control
individual simulations (Keating et al., 2003). Although individual modules within APSIM have been validated extensively, the model has never been used to simulate a lysimeter experiment under conditions of concurrently applied urine and fertiliser to determine the fate of N through all potential pathways (leaching, plant uptake, and gaseous emissions) in a pastoral system. Furthermore, additional validations of APSIM are always useful for general model evaluation and identifying problems, if they exist.

2. The second key objective was therefore to (a) run an APSIM simulation that directly mimicked the experimental conditions of the lysimeter study in Objective 1 above, (b) validate the modelled output data against the experimental data, and (c) determine, based on the relative agreement between the modelled and experimental data, whether the APSIM simulation is appropriate for extrapolative analyses of the lysimeter experimental data.

The design and implementation of the lysimeter experiment encouraged further consideration as to how accurately urine patch dynamics are accounted for in lysimeter studies, and whether they differ in any way from urine patches in the field. It was considered that the key point of difference between a urine patch in a lysimeter and a urine patch in the field is the edge effects, due to the barrier of the lysimeter casing to the surrounding soil and pasture. This raised questions as to whether the ‘effective area’ of a urine patch was accounted for in lysimeter studies. The effective area of a urine patch consists of two areas (a) the wetted area, where urine is directly deposited, and (b) the area outside the wetted area that can access the urinary N, and has previously been estimated to be more than double the size of the wetted area (Lotero et al., 1966; Lantinga et al., 1987; Deenen and Middelkoop, 1992; Moir et al., 2011). However, there is very little data quantifying the effective area of urine patches. While many visual assessments have established that the effective area is in fact larger than the wetted area (Deenen and Middelkoop, 1992; Moir et al., 2011), few have measured and/or partitioned the urinary component of N uptake by pasture and N dynamics in the soil.

Commonly in lysimeter studies, urine is applied over the whole lysimeter surface area (Clough et al., 1998b; Silva et al., 1999; Di and Cameron, 2002a, 2007); therefore, due to the barrier imposed by the lysimeter casing, the effective area does not exist, only the wetted area, which may, in turn, affect the partitioning of N in the lysimeter differently to what would occur in a field scenario. Furthermore, this may have implications for the accuracy of agricultural models that are parameterised and validated based on experimental lysimeter data.
3. The third key objective was therefore to quantify the extent of the effective area of a urine patch on pasture response and soil N dynamics.

This thesis is structured into six chapters, with this being the introductory chapter. Chapter two summarises the current state of knowledge on N dynamics and the fate of urinary and fertiliser derived N in pastoral systems. Chapters three, four and five address objectives one, two and three above, respectively. Chapter six concludes by summarising the findings of the three previous chapters and discusses the limitations and implications associated with them, along with recommendations for future work. Further information is included as appendices including APSIM’s module descriptions (Appendix A) and the details and results of a sensitivity study carried out in APSIM (Appendix B).
Chapter 2
Literature review

2.1 Introduction

The purpose of this review was to evaluate and synthesise the literature in order to highlight gaps in the current understanding of how fertiliser and ruminant urine-N interacts within pastoral systems. This review covers:

- An introduction to the global importance of N;
- The key processes behind soil N transformations in pastoral systems and the subsequent fate of this N;
- Why the fate of N is an important environmental problem;
- The role of urinary-N returns and fertiliser-N application in the cycling and fate of N in pastoral systems, and the interaction between the two when they are deposited simultaneously;
- The background to the role of process based models in aiding agricultural science.

The research gaps identified in this literature review form the foundation for new research objectives.

2.2 The agricultural N cycle

2.2.1 The global importance of N in agriculture

Nitrogen (N) is an essential element for the growth of plants and animals and is the most abundant of all the fundamental macronutrients (N, C, P, O, S) (Galloway et al., 2003). Ironically, N is also a major limiting factor for growth in both natural and agricultural ecosystems (Vitousek et al., 1997) because although it is naturally abundant, it is almost entirely (>99%) in the diatomic N$_2$ form, which is unavailable to most (>99%) living organisms (Galloway et al., 2003). Severing the triple bond, and converting the non-reactive N$_2$ to reactive forms requires a great deal of energy that can only be achieved by specialised N-fixing micro-organisms, and in high temperature processes (Galloway et al., 2003).
Human activities including the combustion of fossil fuels, production of N fertilisers, and cultivation of N fixing legumes, along with population and industry growth, have significantly increased the availability and mobility of N worldwide (Vitousek et al., 1997; Sutton et al., 2011). This has caused reactive N to accumulate in the environment at all spatial scales (Galloway et al., 2003). Perhaps the most significant anthropogenic alteration of the N cycle results from the development of the Haber-Bosch process in the early 20th century (Sutton et al., 2011). The Haber-Bosch process synthesises ammonia by reacting N\textsubscript{2} and hydrogen (H) using an enriched iron or ruthenium catalyst (Smil, 2001). Industrial scale N fertiliser production using the Haber Bosch process began in the early 1940s and has increased exponentially ever since.

On a global scale, around 75% of the 165 teragrams (Tg) of reactive N produced each year is related to agriculture (Galloway et al., 2003). While the use of N fertilisers has led to enhanced food production and sustained population growth, the accumulation of reactive N in the environment over time has been to the detriment of the environment (Sutton et al., 2011). Reactive N is partly responsible for the accelerated eutrophication of many surface water ecosystems, leading to loss of biodiversity and habitat degradation (Galloway et al., 2003). It also indirectly contributes to global climate change (e.g. nitrous oxide (N\textsubscript{2}O) emissions) and stratospheric ozone depletion, and (along with S) to the acidification of lakes and streams in some regions (Vitousek et al., 1997). In the atmosphere, reactive N can also contribute to the production of smog, tropospheric ozone and aerosols, which can cause health problems for humans.

### 2.2.2 Soil N in pastoral systems

New Zealand soils generally contain between 0.1 and 0.6% N in the top 15 cm, the majority of which (>95%) is present in soil organic matter (decomposing plant material, humus and microbial biomass) and not immediately available for plant uptake (Haynes, 1986b). Soil inorganic N, consisting of nitrate (NO\textsubscript{3}^-N), nitrite (NO\textsubscript{2}^-N) and ammonium (NH\textsubscript{4}^+-N) represents a small and transient N pool that accounts for < 2% of the total soil N content (Haynes, 1986b) and is directly available for plant uptake. The processes governing the transformation of N forms in soil are vital to understanding the partitioning of N in the soil-plant system and its fate. The agricultural N cycle is described in detail by Haynes (1986b), Whitehead (1995), and McLaren and Cameron (1996) among others,
and is summarised in this chapter. A generalised diagram of the N cycle is shown below (Figure 2.1).

![Diagram of the soil nitrogen cycle](image)

**Figure 2.1 The soil nitrogen cycle (Di and Cameron, 2002b).**

### 2.2.3 Nitrogen inputs

Inputs of N to soil in pastoral dairy systems include fertiliser application, excreta from grazing animals (urine and dung), effluent application to land and biological N fixation (BNF) by legumes. Urinary N and fertiliser inputs will be discussed in detail in Section 2.4 and Section 2.5, respectively. Other minor N inputs may include wet and dry deposition, and the weathering of soil parent material.

While atmospheric N deposition is a major N input in other areas of the world, it is not so in New Zealand due to less intensive industrial and animal production systems. New Zealand is also a small island nation surrounded by ocean with prevailing westerly wind patterns and therefore suspended N tends to be blown out to sea rather than deposited on land (Parfitt *et al.*, 2006). Wet N deposition from rain generally occurs in industrialised areas with high atmospheric levels of N oxides (Cameron, 1992). There are few such
areas in New Zealand and as such, wet deposition in is estimated to be only 1-2 kg N ha\(^{-1}\) yr\(^{-1}\) (Nichol et al., 1997).

In New Zealand, dairy shed effluent typically comprises a mixture of approximately 10% dung and urine, diluted with wash-down water, with an N concentration of 140-670 mg N L\(^{-1}\) and a dry matter (DM) content not exceeding 5% (Di et al., 1998; Longhurst et al., 2000). Effluent is typically stored for primary treatment in a pond system before being spread over the land. Unlike direct animal excreta returns, it does not introduce high concentrations of N to the soil because it is diluted and evenly spread.

Biological fixation of atmospheric N\(_2\) is an important source of N in many agricultural systems and is carried out by prokaryotic organisms living freely or in symbiotic association with plants (Haynes, 1986b). The enzyme complex dinitrogenase in prokaryotic organisms catalyses the reduction of N\(_2\) to NH\(_3\) (Hopkins and Huner, 2009). In pastoral systems, N fixing bacteria (e.g. rhizobia) and leguminous plants (e.g. clover) form specific associations where nodules are formed on the roots of the host plant. Bacteria live in the root nodules and fix atmospheric N\(_2\), which can subsequently be utilised by the plant. In New Zealand dairy systems, BNF in grass/clover swards is estimated at around 140 kg N ha\(^{-1}\) per year (Ledgard et al., 1999) but can range between 100 and 300 kg N ha\(^{-1}\) (Ledgard et al., 1990). Inorganic N inputs from other sources (e.g. fertiliser and urine deposition) decreases biological N\(_2\) fixation. There are two main reasons for this; firstly, under high soil inorganic N conditions, legumes switch to uptake of inorganic N rather than fixing N\(_2\) because of the high energy demand for N\(_2\) fixation (Hopkins and Huner, 2009); and secondly, after N fertiliser application, the high soil inorganic N conditions promote grass growth, which eventually outcompetes the clover (Ledgard, 2001). Ledgard et al. (1999) showed that in a grass/clover pasture, under 0 N fertiliser application, annual N\(_2\) fixation inputs ranged from 99-231 kg N ha\(^{-1}\), but under fertiliser application of 400 kg N ha\(^{-1}\) yr\(^{-1}\) this declined to 15-44 kg N ha\(^{-1}\).

### 2.2.4 Mineralisation and immobilisation

Mineralisation is the microbially mediated conversion of organic N forms to mineral N (inorganic) forms. Mineralisation involves a series of reactions that breakdown complex proteins into amino acids, ultimately, releasing ammonia (NH\(_3\)) (Equation 2.1). The final stage, where NH\(_3\) is released, is known as ‘ammonification’ and provides energy for the micro-organisms, as well as a source of N (Cameron, 1992). Ammonification is carried
out by a wide range of heterotrophic microorganisms, primarily under aerobic conditions, using organic material as an energy source and O₂ as an electron acceptor (Cameron, 1992). Mineralisation therefore occurs most rapidly in well aerated soils.

\[ R - NH_2 + H_2O \rightarrow NH_3 + R - OH + energy \]  

Immobilisation is the opposite process to mineralisation whereby mineral N is incorporated into microbial pool (Haynes, 1986b). Mineralisation and immobilisation processes occur simultaneously in the soil and typically, when more mineral N is released than is immobilised, ‘net mineralisation’ occurs, and when more mineral N is incorporated by microbes than released by mineralisation, ‘net immobilisation’ occurs. One of the main factors limiting the mineralisation rate is the amount of carbon (C) available relative to N (C:N ratio) (Chadwick et al., 2000). Soil microbes undertaking decomposition only require a certain amount of N relative to C, so any excess N is released and becomes mineralised (Haynes, 1986b). By contrast, when decomposing organic matter has a low N content, there is plentiful energy in the form of C but insufficient N, so the microbes take up mineral N from the surrounding soil (Haynes, 1986b).

Other factors influencing mineralisation/immobilisation rates include temperature: warmer temperatures increase microbial activity, as well as freezing and thawing cycles, which have been shown to stimulate decomposition (Gasser, 1958; Haynes, 1986b); soil moisture content: wetting and drying cycles increase available substrate (Haynes et al., 1986); cultivation and earthworm activity: soil substrate is broken up, increasing microbial surface area exposure to oxygen (Haynes, 1986b; Lovell and Jarvis, 1998); and soil pH: treatment of acid soils with lime stimulates organic matter decomposition (Alexander, 1977).

### 2.2.5 Nitrification

Nitrification is the two-step biological oxidation of NH₃ to nitrite (NO₂⁻) and thereafter, nitrate (NO₃⁻) (Delwiche, 1981). In the first step (Equation 2.2), ammonia oxidising bacteria, including the genera *Nitrosomas*, *Nitrosolobus* and *Nitrospira*, oxidise NH₄⁺ to NO₂⁻, catalysed by the ammonia monoxygenase enzyme associated with the bacteria (Wood, 1986; Cameron et al., 2013). In the second step (Equation 2.3), NO₂⁻ is oxidised
to NO$_3^-$ by a group of nitrifying bacteria *Nitrobacter*, catalysed by the nitrite oxidoreductase enzyme (Bock et al., 1986).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$$  \hspace{1cm} 2.2

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy}$$  \hspace{1cm} 2.3

Heterotrophic microorganisms are also known to nitrify NH$_3$ in conditions normally unsuitable for autotrophic nitrifiers (e.g. waterlogged soils or extreme pH) (Haynes, 1986b). Heterotrophic nitrification is considered to be more common among fungi than bacteria; however, some heterotrophic bacteria can nitrify (Wrage et al., 2001) and often denitrify as well. The substrate, intermediates and products of heterotrophic and autotrophic nitrification are the same, however, the enzymes involved are different (Wrage et al., 2001).

Nitrification releases one H$^+$ ion per NO$_3^-$ ion produced, which can result in soil acidification (Haynes, 1986a). It has been suggested that NH$_3$-oxidising archaea may play an important role in nitrification in agricultural soils (Leininger et al., 2006). However, research by Di et al. (2009) showed that under the addition of an NH$_3$ substrate to grassland soil, the activity and numbers of abundant NH$_3$-oxidising archaea communities did not change, while the numbers and activity of NH$_3$-oxidising bacteria increased considerably, thus suggesting that nitrification is largely driven by NH$_3$-oxidising bacteria populations and not the archaea.

Nitrifying bacteria are reliant on NH$_4^+$ or NO$_3^-$ as an energy source so the substrate concentration in the soil can limit the rate of nitrification. The NH$_4^+$ production rate (ammonification rate) also limits nitrification activity (Haynes, 1986a). High concentrations of soil NH$_4^+$ (maximum of 400-800 $\mu$g N g$^{-1}$ soil) can cause NH$_3$ toxicity and depress nitrification rates (Haynes, 1986a). High concentrations of soil NO$_3^-$ (2500-4200 mg N L$^{-1}$) can also depress the nitrification rate (Painter, 1977). The optimal pH for nitrifying bacteria is between pH 7-9. Above pH 7.5, toxic levels of NH$_3$ may inhibit nitrification, while the lower limit is around pH 4.5 (Sarithchandra, 1978). Soil moisture affects nitrification and optimal rates occur in soils at or near field capacity (-10 to -33 kPa) (Malhi and McGill, 1982). In saturated soils, nitrification stops completely as there is insufficient oxygen and in drier soils the nitrification rate slows but upon rewetting there is a characteristic flush of mineralisation, followed by a flush of nitrification.
Optimal soil temperature is 25-35°C (Haynes, 1986a). The minimum and maximum temperatures for nitrification are around 5 and 40°C respectively but this varies depending on climate and soil type (Mahendrappa et al., 1966). Nitrifying bacteria are sensitive to nutrient deficiencies (Purchase, 1974), high levels of trace elements (e.g. chromium, cadmium, copper and aluminium) (Haynes, 1986a) and pesticides (Schmidt, 1982), many, of which, are highly effective at the short term inhibition of nitrification.

2.2.6 Plant uptake

Pasture plants primarily use inorganic N, both NH$_4^+$ and NO$_3^-$ forms. However, NO$_3^-$ must be reduced back to NH$_4^+$ once inside the plant, thus requiring higher energy inputs and thereby reducing the efficiency of plant N utilisation (Haynes, 1986a). On the other hand, because negatively charged NO$_3^-$ is repelled by most soil particles, it is more mobile than NH$_4^+$, and is often more available for uptake by plants (Haynes, 1986a). Plants can also take up some organic N compounds directly via their root systems, or in association with certain types of mycorrhizal fungi (Nasholm et al., 1998; Hodge et al., 2000; Harrison et al., 2008). Plants can also absorb N (primarily gaseous NH$_3$) through their leaves (Sommer and Jensen, 1991; Whitehead, 1995). This contributes to only a small proportion (< 5%) of total plant N uptake. Emission of NH$_3$ from plant leaves can also lead to N losses (Asman, 1998; Ross and Jarvis, 2001; van Hove et al., 2002).

Pasture production estimates from grazed pastoral land, used for dairy farming in the Waikato region of New Zealand, are estimated at 15-17.5 tonnes of DM ha$^{-1}$ yr$^{-1}$ as pasture ingested (Glassey et al., 2010). Depending on the soil type, climatic conditions, fertiliser and grazing regimes, pastures can take up 200–700 kg N ha$^{-1}$ yr$^{-1}$ (Whitehead, 1995). On a dry weight basis, plant material usually contains between 1 and 4% N (Hopkins and Huner, 2009). Under conditions of high N availability, plants may also take up N in excess of their requirements, termed ‘luxury N uptake’, and store the N as NO$_3^-$ or amides (Whitehead, 1995).

When pasture is consumed by dairy cattle, only 20-25% of the dietary N is retained and utilised for the production of milk or weight gain (Whitehead, 1995). The majority of the plant N consumed by dairy cows (60-90%) is excreted (Haynes and Williams, 1993; Di and Cameron, 2002b) and is thus re-introduced to the soil-plant system.
Nitrogen uptake by perennial ryegrass (*Lolium perenne* L.) is influenced by temperature, sunlight hours, soil moisture and the amount of plant available N in the soil. The optimum temperature for pasture growth is in the range of 15-18°C (Mitchell, 1956) with a minimum of 5°C and a maximum of up to 35°C (Whitehead, 1995). Longer sunlight hours allow for greater plant uptake of N due to greater capacity for photosynthesis (Mitchell and Lucanus, 1962). Plants tend to increase their N uptake with increasing inorganic N availability in the root zone, up to a certain point (as shown in Figure 2.9). The responses of grass swards to different rates of N fertiliser application have been studied extensively, usually in trials where the fertiliser is applied in 3-7 even applications during the growing season (Whitehead, 1995). The results show that herbage yield increases considerably with increasing fertiliser application rate, up to approximately 300 kg N/ha, and at application rates above this, the herbage response per kg of fertiliser N applied declines, along with the cost:benefit ratio of the fertiliser (Whitehead, 1995).

### 2.2.7 Ammonia volatilisation

Ammonia volatilisation is the emission of gaseous NH$_3$ from the soil. From an agricultural production perspective, NH$_3$ volatilisation is undesirable because it can represent a significant loss of N from the soil-plant system that could otherwise contribute to increased productivity. The concentration of NH$_4^+$ relative to NH$_3$ is dependent on soil pH, with higher pH favouring the production of NH$_3$ as illustrated by Equation 2.4 below (Cameron *et al.*, 2013):

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \quad 2.4$$

Upon the application of urea fertilisers and the deposition of ruminant urine, the urea is hydrolysed by the ubiquitous urease enzyme to produce ammonium carbonate ((NH$_4$)$_2$CO$_3$). The carbonate ions are then hydrolysed through a series of biochemical and physiochemical processes to produce hydroxide (OH$^-$) and bicarbonate (HCO$_3^-$) ions (Avnimelech and Laher, 1977; Sherlock and Goh, 1984; Saggar *et al.*, 2012) as outlined in Equation 2.5 below (Cameron *et al.*, 2013):

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3 \rightarrow NH_4^+ + NH_3 \uparrow + CO_2 + OH^- \quad 2.5$$

The generation of the OH$^-$ and HCO$_3^-$ ions results in a temporary increase in pH, favouring the forward reaction of Equation 2.4 (Saggar *et al.*, 2004) resulting in NH$_3$ volatilisation.
The factors affecting NH$_3$ volatilisation have been reviewed previously by Haynes and Sherlock (1986), Bussink and Oenema (1998), Sommer et al. (2004) and Cameron et al. (2013). The rate of hydrolysis, and therefore NH$_3$ volatilisation increases with soil temperature from 0-40°C (Saggar et al., 2012). The other main factors affecting NH$_3$ volatilisation include evaporative loss of soil water (Sherlock and Goh, 1984), soil moisture and texture (Ernst and Massey, 1960), wind speed (Watkins et al., 1972; Haynes and Sherlock, 1986), plant cover, the presence of soluble cations and organic matter (Zantua et al., 1977), and also agronomic factors such as the rate of fertiliser application, liming, stocking rates and irrigation schedules (Freney and Black, 1988).

2.2.8 Leaching

The term ‘leaching’ describes the movement of N through the soil profile via drainage water, to below the root zone, where it can no longer be utilised by plants, and ultimately into a groundwater and/or surface water system. The main form of N leached is NO$_3^-$-N, although small amounts of NH$_4^+$-N can also be leached. Another, less documented form of N leached is dissolved organic N (DON) (see Section 2.2.8.2), which can represent a considerable proportion of the total N load leached (van Kessel et al., 2009).

2.2.8.1 Nitrate leaching

The extent of NO$_3^-$-N leached is determined by (a) the concentration of NO$_3^-$-N in the soil solution and (b) the amount of drainage that occurs through the soil over a given period of time. The amount of NO$_3^-$-N in the soil depends on the amount of N (if any) applied, the nitrification rate, the denitrification rate, and the rate of plant N uptake and immobilisation (Cameron et al., 2013). Drainage occurs when soils approach or exceed field capacity, when there is excess rainfall over evapotranspiration. This can occur at any time of the year but usually occurs in the late autumn and winter months. Nitrate is negatively charged, and repelled by the bulk of negatively charged soil thus making it very mobile in the soil.

Nitrate in the drainage water is transported through the soil via a combination of three primary mechanisms: (a) convection, where dissolved NO$_3^-$-N moves with the mass flow of water in the soil, otherwise known as ‘piston displacement’; (b) diffusion, where the uneven distribution of NO$_3^-$-N in solution results in a concentration gradient and consequently the movement of NO$_3^-$-N from highly concentrated areas, to areas of lower concentration; and (c) hydrodynamic dispersion, which is the mixing of soil solute by the
mechanical action of water flow through the soil. This is due to the heterogeneous nature of soil, namely the large variation in soil pore size and thereby pore water velocities, and the tortuosity of soil pores, generating an array of flow paths (Cameron et al., 2013). Collectively, these NO$_3$-N transport mechanisms are termed ‘combined convective-diffusive-dispersive transport’.

When large amounts of water are applied to the soil surface (e.g. heavy rainfall or irrigation), and the deposition rate exceeds that at which water infiltrates the soil matrix, the preferential flow of water through surface connected macropores (> 30 $\mu$m diameter) can occur (Cameron and Haynes, 1986). Earthworm activity, freezing and thawing cycles, and root growth all lead to the development of macropore networks in the soil. When NO$_3$-N is present in the percolating water, the macropore flow can lead to rapid and extensive NO$_3$-N leaching, however, when NO$_3$-N is located within soil aggregates, the drainage water may bypass it through the macropores, resulting in a slower rate of leaching, or less N leached overall.

Season and climate have a large effect on NO$_3$-N leaching with the largest NO$_3$-N losses occurring during winter, when drainage is greatest, pasture uptake is low, and rainfall is in excess of evapotranspiration. Leaching is generally minimal in summer; however it may occur via macropore flow under heavy rainfall events. Soil NO$_3$-N will typically accumulate in the soil over a dry summer due to limited soil water and poor crop uptake and can be leached over the following winter if it is not taken up by plants or immobilised beforehand. Nitrate is less prone to leaching in spring, with warmer temperatures and increasing daylight hours promoting plant N uptake (Cameron and Haynes, 1986).

Soil type can have a large influence on NO$_3$-N leaching with greater losses observed in sandy, poorly structured soils with lower water holding capacity, as opposed to coarsely structured clay soils that are characterised by slower water movement and greater denitrification potentials (Cameron and Haynes, 1986; Di and Cameron, 2002b). The organic N status of the soil and mineralisation rate can also affect NO$_3$-N leaching. The amount of soil N mineralised will vary significantly depending on environmental conditions and land management (Cameron and Haynes, 1986).

Nitrate leaching is also affected by the N inputs and management of pastoral systems. Nitrate leaching from undisturbed or extensively managed ecosystems is typically very
Nitrate leaching generally increases in grazed pasture systems because most of the N ingested by animals (60-90%) is returned to the soil as excreta in small concentrated patches (Jarvis et al., 1995). A grazing dairy cow can potentially excrete up to 1200 kg N ha$^{-1}$ in a single urine patch (Haynes and Williams, 1993; Di and Cameron, 2002b). Some of this urinary N will be volatilised as NH$_3$, but most will be nitrified to NO$_3^{-}$-N. This N loading rate is far in excess of what the affected pasture can utilise, therefore the unused NO$_3^{-}$-N remains in the soil. Some of this may be denitrified or immobilised, but the majority is leached upon the onset of drainage. In grazed pastoral systems, increased stocking rates increase the frequency of urination events and therefore urinary induced NO$_3^{-}$-N leaching. Timing and rate of N fertiliser and effluent applications are also important. If fertiliser and/or effluent are applied in excess of pasture requirements, or are applied during periods of saturated conditions or drainage, the rates and amounts of NO$_3^{-}$-N leaching increase considerably (Cameron and Haynes, 1986; Di and Cameron, 2002b). The effects of ruminant urine deposition and fertiliser N application are discussion in further detail in Sections 2.4.3 and 2.5.1, respectively.

**2.2.8.2 Dissolved organic N leaching**

Dissolved organic N typically comprises 0.15-0.19% of the total N in agricultural soils (Haynes, 2000). Dissolved organic N is formed in agricultural soils as part of the decomposition process, deriving from soil organic matter and crop residues (van Kessel et al., 2009).

Van Kessel et al. (2009) depict DON as a significant pathway of N loss from agriculture, however, it is largely overlooked in leaching studies, and is often unaccounted for or given little attention in agricultural simulation models, and nutrient budgeting. Van Kessel et al. (2009) surveyed 16 studies which reported both DON and NO$_3^{-}$-N leaching losses, and of these, only one study (Lawes et al., 1881) was published prior to 2000. Of the studies in this review, the lowest DON losses were 0.3 kg N ha$^{-1}$ yr$^{-1}$ from a sandy loam soil under inorganic N inputs of 220 kg N ha$^{-1}$ yr$^{-1}$, and the greatest DON losses were 127 kg N ha$^{-1}$ yr$^{-1}$ from lysimeters filled with sand and an intact pasture sod treated with 1030 kg N ha$^{-1}$ yr$^{-1}$ urine (Wachendorf et al., 2005). Overall, DON losses averaged 12.7 kg N ha$^{-1}$ yr$^{-1}$ across all treatments and sites and the general trend was for DON to increase with increasing N inputs, particularly urine application (van Kessel et al., 2009). Furthermore, DON loss contributed on average, 26% of the total soluble N loss, and constituted nearly one third of the NO$_3^{-}$-N leaching losses. With the exception of the
above mentioned studies identified by van Kessel (2009), there remains a considerable
dearth of understanding surrounding DON leaching from agricultural systems.

Like NO$_3^-$-N leaching, DON leaching is controlled primarily by drainage as a result of
precipitation or irrigation events. The extent of DON leached depends on the magnitude
and frequency of these drainage events, and the length of time since the soil profile was
last flushed (Cooper et al., 2007). The longer the antecedent pre-drainage period, the
more biologically derived soluble organic matter can accumulate in the soil (Cooper et
al., 2007). Even with large amounts of accumulated DON in the soil, leaching requires
sufficient precipitation/irrigation to induce drainage. Conversely, with little or no
accumulated DON in the top soil, precipitation/irrigation events will result in low DON
losses.

The deposition of urine introduces soluble N directly to the soil and also, perhaps more
importantly, the resulting rise in soil pH in the urine patch increases the solubilisation
of soil organic matter, further increasing soil DON (Wachendorf et al., 2005). The
frequency of urine deposition events and therefore stocking rate can have a considerable
impact on the DON leaching losses.

Soil microbes use DON as a substrate, and microbial activity has been shown to decrease
the concentration of DON in the soil (van Kessel et al., 2009), with the more labile
fractions of DON being preferentially metabolised, changing its composition and its
availability for leaching as it moves through the soil profile (Lajtha et al., 2005).

2.2.8.3 Measuring nitrate leaching

Some of the common methods available for measuring NO$_3^-$-N leaching from pastoral
land on a plot, paddock or farm scale include include lysimeters, ceramic suction cups,
and drainage plots (Weihermüller et al., 2007).

Lysimeters are undisturbed monoliths of soil contained within a cylindrical casing used
for the collection and integrated measurement of the amount and composition of drainage
water in a known soil volume (Cameron et al., 1992). They may be installed in the field,
or collected and transported to purpose built research facilities. A widely used method
for collecting lysimeters is detailed by Cameron et al. (1992). Advantages of lysimeters
include very accurate measurement of drainage and solute concentration, while permitting
a wide range of treatment comparisons, along with gas flux measurements from the soil
surface. Their limitations include limited depth in relation to the field soil pore system, unsuitability for sloping land, and the high cost and labour required for collection and installation.

Porous ceramic suction cups are buried at a 45° angle in the soil and a vacuum is applied to extract soil water into the cup. Assembly and installation of suction cups is described by Wood (1973), and more recent developments are described by Lord and shepherd (1993). The main advantages of suction cups are that they are inexpensive per unit, and can be installed in situ in large numbers, allowing for replication and direct measurement from grazed pasture. Their disadvantages include the fact that they are unsuitable in coarse textured soils can only be used to measure the solute concentration in a small sample of soil solution. As a result, very large replication is required for representation at the field or paddock scale, and they provide no indication of the amount of drainage.

Drainage plots are most appropriate in soils that have an impermeable clay layer. They allow for the measurement of drainage volume and NO3-N concentrations from a defined area. Their main advantage is that stock can graze the surface and deposit urine and dung under true field conditions. However, stock may camp on the drainage plot sites, or avoid it all together, resulting in unrepresentative leaching results. The major disadvantage of drainage plots is that drainage tiles commonly intercept only part of the water flow down the soil profile, and therefore may misinterpret true drainage and leaching losses (Cameron and Haynes, 1986).

2.2.9 Denitrification and N2O emissions

Nitrous oxide is produced in soil by both biological and non-biological processes. Biological processes are estimated to account for more than 95% of global N2O production (Müller, 1995). Of the biological processes that produce N2O, denitrification is arguably the most important.

Denitrification is the biological reduction of NO3− and NO2− to volatile gases, namely NO, N2O and N2. It is a respiratory process carried out by denitrifying bacteria (predominantly heterotrophs) which obtain their energy and cellular C from the oxidation of organic substrates under anaerobic conditions using N-oxides as terminal electron acceptors (Haynes and Sherlock, 1986). Under anaerobic conditions, in the presence of
NO₃⁻, denitrifying bacteria undergo NO₃⁻ respiration which is defined in Equation 2.6 below (using glucose as an example of a carbohydrate) (Broadbent and Clark, 1965):

\[
C_6H_{12}O_6 + 4NO_3^- \rightarrow 6CO_2 + 6H_2O + 2N_2
\]  

2.6

The denitrification process occurs as a sequence of steps with the progressive loss of O₂. Each step requires a reducing enzyme (Figure 2.2). Not all denitrifying bacteria possess the full suite of enzymes required for complete denitrification, and as a result NO and N₂O gases can be released, with N₂O and N₂ being the dominant products (Haynes and Sherlock, 1986).

![Figure 2.2 General pathway sequence of denitrification (Haynes and Sherlock, 1986). Values in brackets denote the oxidation state of N.](image)

Denitrification may cause a considerable loss of N from pastoral systems. Emissions of N₂O are of particular concern due to increasing atmospheric concentrations and the fact that N₂O is a potent greenhouse gas (GHG). Furthermore, agriculture is the largest global anthropogenic N₂O source (Kroeze and Mosier, 2002), and due to New Zealand’s uniquely large agricultural sector, its N₂O emissions constitute a large proportion (17.2%) of its total GHG inventory (Ministry for the Environment, 2012). The implications of this are discussed later in Section 2.3.2.

Denitrification rates in soil are affected by a range of factors. The most important being the fluctuation of the partial pressure of O₂. Denitrification occurs under anaerobic conditions and the activity of all N oxide reductase enzymes are repressed by the presence of O₂. Soil moisture has a large effect on the O₂ status in soil: saturated soils produce favourable anaerobic conditions for denitrification, however, denitrification also occurs at anaerobic microsites in unsaturated soil e.g. in the centre of soil aggregates (Firestone, 1982; Knowles, 1982; Carter, 2007).

Another important regulator of denitrification rates is the availability of organic C (Šimek et al., 2002). Most denitrifying bacteria are heterotrophs, which require organic compounds as electron donors and as a source of cellular material (Tiedje, 1988). Large
N₂O fluxes have been observed during alternate wetting and drying cycles (Firestone and Tiedje, 1979). This is most likely due to changes in the availability of soil organic matter and microbial activity with alternating dry and wet conditions, e.g. under dry conditions, soil shrinkage and microbial death can increase soil organic matter, and upon rewetting, a flush in microbial activity occurs, often resulting in a pulse of N₂O emissions.

The concentration of NO₃⁻-N substrate also affects the denitrification rate. If soil NO₃⁻-N concentration is low, the capacity for denitrification, and thereby N₂O production is limited. High concentrations of soil NO₃⁻-N have also been shown to inhibit N₂O reductase during denitrification (Firestone and Tiedje, 1979), which increases the ratio of N₂O:N₂ produced.

Many other factors influence the denitrification rates, including soil texture and structure, soil biological activity, plants, rainfall, temperature and pH (Šimek et al., 2002). There is a large amount of spatial and temporal variability in these factors and they all directly or indirectly affect the main regulators of denitrification outlined above. At temperatures of 10-35°C denitrification rates can increase 2 fold for every 10°C increase in temperature and continue to increase up to a maximum of 65-70°C (Dawson and Murphy, 1972). Denitrification rates slow considerably below 10°C (Haynes and Williams, 1993). The optimum pH range for denitrifiers was initially reported as 7.0-8.0 (Nommik, 1956). However, Šimek et al. (2002) showed that the optimum pH can shift as populations of soil denitrifiers adapt to the prevailing soil pH. Also, in the alkaline region of the pH scale, N₂ becomes a significantly more prominent product of denitrification than N₂O, as illustrated by Figure 2.3 below.

![Figure 2.3 Denitrifying enzyme activity at natural and modified soil pHs showing N₂ and N₂O production (Šimek et al., 2002).](image)
There is considerable temporal variability in soil N$_2$O emissions, both diurnally and seasonally. Diurnal changes are primarily due to changes in temperature (Christensen, 1983), with the highest emissions corresponding with the late afternoon, following the warmest time of the day. Christensen (1983) showed that diurnal fluctuations in N$_2$O evolution were more obvious when irradiation was high, and Das et al. (2012) showed diurnal fluctuations were largest when temperature differences throughout the day were large. Seasonal differences in N$_2$O emissions can be attributed to changes in soil moisture status and temperature with seasonal climatic change.

High spatial variation in N$_2$O emissions is also reported, from microscales to regional scales (Groffman and Tiedje, 1989; Hénault et al., 2012). Variation at smaller scales (< 1 m$^2$) is often greatest due to soil heterogeneity and the uneven distribution of anaerobic microsites resulting in “hot spots” of N$_2$O evolution (van den Heuvel et al., 2009) which may account for a significant proportion of the total flux. At larger scales spatial variability can be linked to changes in soil type, NO$_3^-$-N availability and topographic effects (Hénault et al., 2012). Furthermore, N$_2$O produced in the soil is not necessarily lost from the system immediately: it can be consumed at another location (e.g. in an overlying soil layer) (van den Heuvel et al., 2009) or trapped in the soil for a length of time before being emitted at a later date (Clough et al., 2005).

Earthworm activity can also cause variability in N$_2$O production. The earthworm gut is an ideal habitat for denitrifiers, leading to N$_2$O emissions from the earthworms themselves (Horn et al., 2003; Bertora et al., 2007). Furthermore, increased denitrification and N$_2$O emissions have been observed from worm casts. Elliot et al. (1990) recorded increases of N$_2$O fluxes up to five times greater from worm casts than background N$_2$O fluxes. Finally, but most importantly, earthworm activity in soil affects soil structure and increases soil porosity (Blouin et al., 2013), increasing the capacity for gas exchange with the atmosphere and reducing the likelihood of N$_2$O being further denitrified to N$_2$ prior to atmospheric exchange (Bertora et al., 2007).

In addition to denitrification, there are alternative pathways that result in the production of N$_2$O, which are discussed below. It is considered that the majority of N$_2$O evolved in soils is produced via either denitrification, nitrification, or nitrifier denitrification and that other pathways contribute very small amounts to the total N$_2$O budget (Bremner, 1997).
a) Nitrification

The nitrification process is detailed in Section 2.2.5. During the oxidation of NH$_3$ to NO$_2^-$ (Equation 2.2), an intermediate hydroxylamine (NH$_2$OH) is produced (Bremner, 1997). The activity of the nitrifying bacteria can produce N$_2$O from both the NH$_2$OH and NO$_2^-$ intermediaries under aerobic conditions (Figure 2.4). It was previously thought that the majority of N$_2$O evolved in aerobic soils is produced via nitrification (Bremner, 1997), however, recent research suggests nitrifier denitrification (discussed next) contributes to more aerobically produced N$_2$O than previously thought (Kool et al., 2011).

![Figure 2.4 Nitrification: pathways and enzymes involved (Wrage et al., 2001).](image)

b) Nitrifier denitrification

Nitrifier denitrification is an alternative pathway of nitrification where the oxidation of NH$_3$ to NO$_2^-$ is followed by the reduction of NO$_2^-$ to N$_2$O and/or N$_2$ (Figure 2.5). The first part of the reaction is nitrification of NH$_3$ to NO$_2^-$ and the second part is denitrification of NO$_2^-$ but the whole sequence of reactions is thought to be carried out by only autotrophic NH$_3$-oxidisers (Wrage et al., 2001). There remains much unknown about the dynamics of N$_2$O production via nitrifier denitrification in pastoral systems, however, research increasingly suggests that this pathway contributes substantially to the total nitrifier-induced N$_2$O production in pastoral soils (Kool et al., 2010; Kool et al., 2011).
Figure 2.5 Nitrifier denitrification theoretical pathway (Wrage et al., 2001).

c) Heterotrophic denitrification

Heterotrophic bacteria can be distinguished by their ability to both nitrify and denitrify; however, in contrast to conventional denitrifiers, they do so under aerobic conditions (Wrage et al., 2001). Although heterotrophic nitrification is typically considered a minor source of N₂O, under aerobic conditions, heterotrophic nitrifiers produce far more N₂O per cell than autotrophic nitrifiers (Anderson et al., 1993).

d) Dissimilatory nitrate reduction (DRNA)

Dissimilatory nitrate reduction to ammonium (DNRA) is where reduction proceeds to produce NH₄⁺ and N₂O is formed as a by-product. This process is dependent on the presence of O₂, but is not affected by NH₄⁺ (Müller, 1995). The fundamental difference between denitrification and DNRA is that N₂ and N₂O are lost from the system, whereas NH₄⁺ is not (Golterman, 2004). This pathway is generally considered to be a minor source of N₂O production (Bleakley and Tiedje, 1982).

e) Assimilatory nitrate reduction

Assimilatory nitrate reduction is the incorporation of NO₃⁻ into the biomass. Bacteria, cyanobacteria and fungi reduce NO₃⁻ to NH₄⁺ (Paul and Clark, 1989) and N₂O is produced as an intermediate by-product of hyponitrite (Freney et al., 1979). Some studies have found this pathway to be of significance under fungal activity in forest soils, e.g. Robertson and Tiedje (1987) identified fungal activity as an alternative biological N₂O source, and a later study by Sextone (1991) reported that N₂O produced as a result of fungal activity could be up to 40% of the total N₂O produced.
Chemodenitrification is a non-biological reaction involving the chemical decomposition of by-products from NH$_4^+$ oxidation to NO$_2^-$ (Wrage et al., 2001). These reactions typically occur at low pH and the primary product is NO, and N$_2$O to a lesser extent (Chalk and Smith, 1983). Chemo-denitrification can become significant in soils that are aerobic, have an organic matter supply and where NO$_2^-$ accumulation occurs (Mosier et al., 1983; Venterea, 2007). Other non-biological mechanisms that produce N$_2$O include the reaction of HNO$_2$ with soil amino compounds (Whitehead, 1995), and the reaction of HNO$_2$ with oxides formed during organic matter composition (Porter, 1969).

### 2.2.9.1 Measuring N$_2$O emissions

There are two main approaches for measuring N$_2$O: chamber and micrometeorological techniques. Static chambers (early methodology is described by Matthias et al. (1980)) are widely used and consist of an open chamber protruding into the soil, with an insulated headspace cover. The chambers have either water baths or rubber seals to create an air-tight seal on application of the headspace cover to the chamber base. The top of the cover has a sample port and headspace gas samples are collected, usually manually using syringes. Recent advanced systems use vacuum pumps or automated flux monitoring systems (Alves et al., 2012; van der Weerden et al., 2013).

Micrometeorological techniques use analyses of the atmospheric concentration of N$_2$O and meteorological variables including wind speed, air temperatures, radiation and heat fluxes to calculate field scale N$_2$O fluxes (Christensen et al., 1996; Phillips et al., 2007).

Chamber techniques are less costly and provide reliable punctual measurements in time and space, but they do not give a reliable representation of field or ecosystem scale N$_2$O fluxes, which is where integrative meteorological techniques become advantageous (Hénault et al., 2012). Also, chamber methods disturb the natural microclimate inside the chamber and changing gas concentrations may affect the flux, whereas micrometeorological techniques account for atmospheric conditions and wind circulation (Hénault et al., 2012).

### 2.3 Effects of the fate of N on the environment

In an ideal pastoral dairy system scenario, the N inputs to the system would be continuously recycled between the soil, plants and animals. However, in more intensive
systems where inputs such as fertiliser and supplementary feed are higher, the risk of N loss from the system via leaching, N₂O emissions and NH₃ volatilisation increases, particularly in grazed systems where urine patches are prevalent. From both a productivity, and environmental perspective, the uptake of N by plants or the incorporation of N into soil organic matter is perceived as a good outcome in the short term. The loss of N as gaseous N emissions or leaching are undesirable outcomes, resulting in net losses of N from the system, representing a loss of soil fertility and potential productivity, as well as a threat to the environment (Dungait et al., 2012). The consequences of these undesirable N losses to the wider environment are discussed below.

2.3.1 Leaching

Nitrate leaching represents a potential threat to human health as well as the wider environment (Di and Cameron, 2002b). The New Zealand Ministry of Health ‘Maximum Acceptable Value’ (MAV) for NO₃⁻ in drinking water is 50 mg L⁻¹, which is equivalent to 11.3 mg L⁻¹ NO₃⁻-N (Hayward and Hanson, 2004). It is advocated that preventative and remedial measures should be undertaken if the NO₃⁻-N concentration in a water supply reaches half of the MAV (5.6 mg L⁻¹). This drinking water guideline pertains primarily to the health risk posed to bottle fed infants where consumption of water with high levels of NO₃⁻ can lead to the development of acute methaemoglobinemia (blue baby syndrome) which reduces the oxygen-carrying capacity of the blood (Hayward and Hanson, 2004). High NO₃⁻-N concentrations in drinking water have also been linked to some cancers and heart disease (Grizzetti et al., 2011).

From an environmental perspective, there is strong evidence to suggest that New Zealand’s lakes, streams and groundwater reservoirs are becoming nutrient enriched as a result of increased NO₃⁻-N loads (Parliamentary Commissioner for the Environment, 2004). Elevated NO₃⁻-N loads into ground and surface water systems can cause accelerated eutrophication. Most surface water bodies in New Zealand are N and/or P limited, therefore the introduction of available NO₃⁻-N to aquatic systems increases productivity, resulting in enhanced algal and macrophytic growth, including toxic algal blooms. The subsequent death and decomposition of the algae and macrophytes can lead to reduced dissolved oxygen concentrations, and in worst cases can eventually cause hypoxia, loss of biodiversity, and habitat degradation. Eutrophic water bodies are also
typically odorous and unsightly, and create problems for recreational activities such as boating, swimming and water sports.

2.3.1.1 Mitigation/management of nitrate leaching

The diffuse nature of N losses from grazed pasture poses a major challenge to reducing NO$_3^-$-N leaching. Non-point sources of N to water bodies (e.g. urine patches) are estimated to contribute > 80% of the total N entering surface and groundwater (Cameron and Haynes, 1986) yet they are one of the most difficult sources to mitigate. As discussed, NO$_3^-$-N leaching is affected by a large number of soil, environmental and management factors. Many of these factors cannot be altered or managed, therefore an integrated approach to on-farm decision making is essential to minimise NO$_3^-$-N leaching and optimise plant N use efficiency so that production is not compromised. Some common mitigation options for reducing NO$_3^-$-N leaching from pastoral systems are outlined below (Di and Cameron, 2002b):

- **Limiting N application rates and synchronising application to plant demand:**
  Ensuring N supply meets plant demand is an effective way to reduce the risk of NO$_3^-$-N leaching. This approach requires an understanding of plant N needs throughout the year, and the ability of the soil to meet that N demand. Required N inputs will vary with each pastoral system; however, generally, application rates of N fertiliser should not exceed 200-250 kg N ha$^{-1}$ yr$^{-1}$ (Di and Cameron, 2002b).

- **Split fertiliser applications:** Smaller and more frequent fertiliser applications, as opposed to fewer large applications, can increase fertiliser N use efficiency by pasture (Addiscott, 1996).

- **Balancing other nutrient inputs:** Other important plant nutrients (e.g. phosphorus and sulphur) may also limit plant growth and thus prevent maximum N use efficiency (Di and Cameron, 2002b). Soil tests indicate nutrient deficiencies if they exist, and these nutrients should be applied rather than increasing N application.

- **Limit N application during high risk seasons:** There is greater risk of NO$_3^-$-N leaching during drainage (usually autumn/winter), when rainfall is high, and evapotranspiration and plant N uptake are low (Whitehead, 1995). Fertiliser or effluent applications should be avoided at these times.
• Use of nitrification inhibitors: The application of nitrification inhibitors (e.g. dicyandiamide, ‘DCD’) inhibits the oxidation of NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{-} in the soil (Di and Cameron, 2005). Positively charged NH\textsubscript{4}\textsuperscript{+} ions are adsorbed onto negatively charged soil particles and are held in the soil profile rather than leached. The effectiveness of nitrification inhibitors varies with soil and environmental factors.

• Stock management: Urine returns from grazing animals can result in the highest leaching losses (Cameron et al., 2013). Nitrate leaching via urine patches can be reduced by effective stock management, e.g. removing stock from pasture during critical times in the autumn/winter months.

• Riparian zones: Riparian planting between productive land and surface water bodies can reduce the NO\textsubscript{3}^-N load entering the water bodies by removing N at the soil surface (Di and Cameron, 2002b).

2.3.2 Nitrous oxide emissions

Nitrous oxide is one of the few gases that is a GHG, and has a role in ozone depletion (Ravishankara et al., 2009). Although N\textsubscript{2}O only accounts for around 0.03% of total global GHG emissions, it has a global warming potential of 298 times that of carbon dioxide, over a 100 year time period (Table 2.1) (IPCC, 2007; Thomson et al., 2012).

<table>
<thead>
<tr>
<th>Gas</th>
<th>Atmospheric Lifetime (years)</th>
<th>Global Warming Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide CO\textsubscript{2}</td>
<td>50 – 200</td>
<td>1</td>
</tr>
<tr>
<td>Methane CH\textsubscript{4}</td>
<td>12 ± 3</td>
<td>21</td>
</tr>
<tr>
<td>Nitrous oxide N\textsubscript{2}O</td>
<td>120</td>
<td>298</td>
</tr>
<tr>
<td>CF\textsubscript{4} (PFC)</td>
<td>50,000</td>
<td>6,500</td>
</tr>
<tr>
<td>C\textsubscript{2}F\textsubscript{6} (PFC)</td>
<td>10,000</td>
<td>9,200</td>
</tr>
<tr>
<td>SF\textsubscript{6}</td>
<td>3,200</td>
<td>23,900</td>
</tr>
</tbody>
</table>

Table 2.1: Atmospheric lifetimes and Global Warming Potential (GWP) values for common GHGs (IPCC, 2007).

Global atmospheric N\textsubscript{2}O abundance has risen from around 270 ppb in pre-industrial times, to 324 ppb in 2011 (IPCC, 2013) and continues to increase at a rate of 0.2-0.3% per year (Nevison, 2000; Ravishankara et al., 2009; Thomson et al., 2012). Much of this increase in atmospheric N\textsubscript{2}O concentration is attributed to the expansion of agricultural land area, and intensification of agricultural practices since the pre-industrial era (Reay et
approximately 62% of global \( \text{N}_2\text{O} \) emissions are estimated to come from soils, with an agricultural contribution of 4.2 Tg N yr\(^{-1} \) (Thomson et al., 2012). Other sources of \( \text{N}_2\text{O} \) include the oceans, industrial activity and vehicular transport (Figure 2.6).

![Figure 2.6 Global sources of \( \text{N}_2\text{O} \) production (Thomson et al., 2012).](Image)

New Zealand is unique in that \( \text{N}_2\text{O} \) makes up 17.2% of its GHG emissions inventory, with an estimated 96% coming from agricultural sources (Ministry for the Environment, 2012). By comparison, agricultural emissions from most other developed nations make up less than 12% of their total emissions (Ministry for the Environment, 2012). This is due to New Zealand’s large agricultural sector, where agricultural products comprise 58% of total merchandise exports and contribute almost 50% of the total GHG emissions (Ministry for the Environment, 2012).

It is considered that the increase in global average temperatures (climate change) in the 20\(^{\text{th}} \) century (~0.6°C) are largely attributed to anthropogenic GHG emissions since the industrial revolution (IPCC, 2007) and further increases in temperature are projected if GHG emissions are not reduced. Some climate change effects are already evident e.g. the melting of the Greenland ice shelf in the arctic region (Stendel et al., 2008) and other anticipated effects include global sea level rise and an increasing frequency of storms and/or droughts in some areas (Michener et al., 1997).

The ‘greenhouse effect’ is a natural one, which without, the Earth’s temperatures would be uninhabitably low (mean -18°C) (Hites, 2007). Greenhouse gases are characterised by either permanent or temporary polarity and infrared radiation from the sun is reflected from the Earth surface back towards space, where it can be absorbed by GHGs in the
troposphere (Kotz and Treichel, 2003). This energy is then re-radiated in all directions – some escapes back to space, however, some is re-radiated back down towards Earth, creating the warming effect (Gribbon, 1996). Nitrous oxide is a very effective GHG that absorbs infrared radiation in spectral windows that other gases cannot (Vitousek et al., 1997).

Nitrous oxide is a precursor to NOx gases which catalytically destroy stratospheric ozone and are therefore a source of stratospheric ozone depletion. Reductions in CFC emissions during the late 19th century have resulted in current anthropogenic N₂O emissions being one of the most significant of all the ozone-depleting substances (Ravishankara et al., 2009).

2.3.2.1 Mitigation/management of N₂O emissions

Similarly to NO₃⁻-N leaching, the major challenge associated with mitigating agricultural N₂O emissions is the diffuse and often indirect nature of emissions from grazed pasture systems.

As outlined above, soil N₂O emissions are affected by a range of environmental factors, many of which cannot be altered or managed, therefore an integrated approach to N₂O mitigation is required. Because the agricultural sector will undoubtedly continue to intensify, it is important to understand the variables controlling N₂O emissions so that effective strategies can be developed to control emissions (Seitzinger et al., 2000). Some common mitigation options for N₂O in pastoral systems include (Di and Cameron, 2002b):

- Limiting N application rates and synchronising N application to plant demand;
- Limiting N application during autumn and winter months in order to avoid denitrification associated N₂O emissions;
- The use of nitrification inhibitors (e.g. DCD) to prevent the oxidation of NH₄⁺ to NO₃⁻ and production of N₂O via nitrification and subsequent denitrification; and
- Stock management to reduce grazing of pasture during autumn/winter months to reduce N₂O emissions from excreta deposited on pasture.
2.4 Nitrogen return as urine

Of the N ingested by ruminant cattle, 60-90% is excreted in small, concentrated patches, with the majority (70-80%) deposited as urine (Haynes and Williams, 1993; Jarvis et al., 1995). Loading rates of N in urine patches have been reported to equal 400-1200 kg N ha\(^{-1}\) (Jarvis and Pain, 1990; Di and Cameron, 2002b; Cameron et al., 2013). Following grazing, this results in an irregular array of small patches containing high N loading (compared to the surrounding soil), which manifest as visible patches of denser, darker green pasture (Figure 2.7). The high N loading on the comparatively small area of pasture means that in most cases, the pasture utilises a small proportion of the N available, leaving the remainder vulnerable to leaching (Cameron et al., 2013). It is therefore not surprising that urine patches are the most significant contributor to N leaching from agricultural soils (Haynes and Williams, 1993; Di and Cameron, 2002b).

![Figure 2.7 Visible urine patches in the field.](image)

The number of cattle urination events over a 24 hour period ranges from 8-12 per day (Petersen et al., 1956; Haynes and Williams, 1993; Jarvis et al., 1995; Moir et al., 2011). The mean volume for a single bovine urination event ranges from 1.6-2.2 L (Haynes and Williams, 1993). The number and volume of urinations are highly variable and increase with increased water intake by the animal, as well as increased water ingested through herbage intake (Doak, 1952). The season and climatic conditions also have a large effect, for example on hot days, the water intake and subsequent urinary output from the animals tends to be much greater than on a cooler day (Betteridge et al., 1986), creating seasonal differences in urine volumes. Some examples of the reported frequency and volume of urine deposited by grazing cattle are shown in Table 2.3.
2.4.1 Urine composition

The composition of animal urine varies depending on the diet and the physiological state of individual animals (Whitehead, 1995). Urine typically contains 4-12% dissolved solid material (much of which is nitrogenous) (Safley et al., 1984). The concentration of N in the urine of pasture grazing cattle varies from day to day, with time of day, and between individual animals (Hoogendoorn et al., 2010). Reported urinary N concentrations from cattle in grazed pasture systems range from 0.9-20 g N L⁻¹ (Haynes and Williams, 1993; Hoogendoorn et al., 2010; Dijkstra et al., 2013). Because the concentration of N in cattle urine is largely determined by the amount of surplus metabolised N to be excreted (as well as urine volume and urine frequency) (Hoogendoorn et al., 2010), increases in the N content of the diet result in increases in the concentration of N in urine (Haynes and Williams, 1993; Dijkstra et al., 2013). The N content of urine also varies depending on the type of feed, the nutrient content of the feed, herbage water content, the amount of water consumed by the animal, climatic conditions and the time of day (Sherlock and Goh, 1984; Haynes and Williams, 1993; Dijkstra et al., 2013). Urea is the dominant form of N in cattle urine constituting 65-90% (Bristow et al., 1992). The remainder is made up of amino acids and peptides including allantoin, hippuric acid, creatinine, creatine and uric acid (Doak, 1952) (Table 2.2). The pH of cattle urine generally ranges from 8.4-8.6 (Sherlock and Goh, 1984; Haynes and Williams, 1992).

Table 2.2 Average nitrogenous constituents of cattle urine (Bristow et al., 1992).

<table>
<thead>
<tr>
<th>Urine N constituent</th>
<th>Content in urine (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>16.21</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>7.18</td>
</tr>
<tr>
<td>Allantoin</td>
<td>2.04</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.36</td>
</tr>
<tr>
<td>Xanthine/hypoxanthine</td>
<td>0.12</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.98</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.80</td>
</tr>
<tr>
<td>Free amino acids</td>
<td>0.78</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.37</td>
</tr>
</tbody>
</table>
2.4.2 Area affected by urine patches

The area of a urine patch can be defined by (a) the wetted area, where urine is directly voided and (b) the area immediately outside the wetted area that can access urinary N through plant root extension and N diffusion through the soil (Lantinga et al., 1987; Tinker and Nye, 2000). Together, these two areas can be collectively termed the ‘effective area’ of a urine patch.

The wetted area covered by a single cattle urination event has been reported to range from 0.16-0.49 m² with an average area of 0.26 m² (Table 2.3) (Petersen et al., 1956; Davies et al., 1962; Hogg, 1968; Richards and Wolton, 1975; Haynes and Williams, 1993). The variation in urine patch size also results in large scale spatial heterogeneity of the soil N concentrations they create (Orwin et al., 2009). The wetted area of a urine patch is determined by the volumes of urine deposited, wind, slope, antecedent soil moisture, and soil physical conditions, i.e. compaction (Patra et al., 2005).

Table 2.3 Examples of frequency, volume, and surface area covered by dairy cattle urinations in grazed pasture systems.

<table>
<thead>
<tr>
<th>Study</th>
<th>Frequency of urinations per day</th>
<th>Volume of single urination (L)</th>
<th>Wetted area covered by single urination (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ledgard et al., 1982)</td>
<td>9.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Lovell and Jarvis, 1996a)</td>
<td>10.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Williams et al., 1999)</td>
<td>11.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Williams et al., 2000)</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>(Haynes and Williams, 1999)</td>
<td>9.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Petersen et al., 1956)</td>
<td>8.0</td>
<td>-</td>
<td>0.28</td>
</tr>
<tr>
<td>(Grayston et al., 2001)</td>
<td>10</td>
<td>2.2</td>
<td>0.19</td>
</tr>
<tr>
<td>(Haynes and Sherlock, 1986)</td>
<td>12.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Prins and Neeteson, 1982)</td>
<td>-</td>
<td>-</td>
<td>0.18</td>
</tr>
<tr>
<td>(Safley et al., 1984)</td>
<td>11.0</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>(Cameron et al., 1992)</td>
<td>10.0</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>(Richards and Wolton, 1976)</td>
<td>-</td>
<td>-</td>
<td>0.49</td>
</tr>
<tr>
<td>(Patra et al., 2005)</td>
<td>-</td>
<td>-</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The ‘effective area’ of a urine patch is estimated to be much higher than the wetted area, ranging from 0.03-1.1 m², with an average area of 0.68 m² (Lotero et al., 1966; Lantinga
et al., 1987; Moir et al., 2011). However, quantitative data on the effective area of urine patches are very limited. A lysimeter study was conducted by Decau et al. (2003), where \( ^{15}\text{N} \) labelled urine patches (wetted area of 0.4 m\(^2\)) were applied to the centre of large 2 m\(^2\) lysimeters, and pasture was collected from (a) the wetted area, (b) an area of 0.68 m\(^2\) around it, and (c) an area 0.97 m\(^2\) around that. The findings reported cumulative pasture uptake of urinary-N to be < 0.5, ~5 and ~20 g N m\(^{-2}\) in the outer, middle and wetted areas respectively. Decau et al. (2003) attributed the pasture urinary-N uptake outside the wetted area to soil N diffusion, concluding that the urinary-N did not diffuse beyond 20 cm from the edge of the urine patch. In a field study, Deenan and Middlekoop (1992) investigated the effect of artificial urine on pasture and found that the effective area was confined to within only 150 mm from the edge of the urine patch.

Lotero et al. (1966) measured plant response from concentric bands around the centre of existing urine spots and found that DM yield decreased linearly from the centre to the periphery of the urine patch, affecting a total of 0.9-1.2 m\(^2\). Moir et al. (2011) observed seasonal variation in the effective area whereby urine affected areas tended to be larger from spring/summer deposited urine, and smaller in winter and autumn. These differences were mainly attributed to rapid winter NO\(_3^-\)-N leaching when soils are draining (less N available for plant uptake) but other factors may have included animal N intake in feed (less feed in winter), animal water intake (less water ingested in winter therefore lower urine volumes), and higher spring/summer pasture growth rates (Moir et al., 2011).

The volume of soil wetted by a urine patch varies with surface area, soil moisture, surface water repellence, surface compaction, microtopography, vegetation cover, slope and wind (Williams and Haynes, 1994). Tracer studies have shown that preferential flow of urine occurs following deposition with the wetting front penetrating as deep as 400 mm (Williams and Haynes, 1994) and with up to 46% of the urine lost beyond the top 150 mm of soil (Williams et al., 1990a). In a later study, Monaghan et al. (1999) measured up to 68% (and an average of 17%) of applied urine below 200 mm, 6 hours following application. Figure 2.8 illustrates an example of the distribution of a bromide tracer down the soil profile following a simulated urine application.
Urine patches are estimated to cover from 4-29% of the grazed pasture area per year (Richards and Wolton, 1976; Williams and Haynes, 1994; Whitehead, 2000; White et al., 2001; Dennis et al., 2011; Moir et al., 2011). Urine patch distribution is uneven and heavily influenced by stock behaviour, for example, animal congregation sites such as sheltered areas, around water troughs, gateways, on ridges or hills, and in areas where hay or silage are fed out receive much higher urine loads than other areas (Haynes and Williams, 1993). Stock management can also influence urine patch distribution over a farm. Urine is also deposited on raceways, stock handling facilities and in the milking yard, so the more time cows spend out of the paddock in these ‘non-productive’ areas of the farm, the less urine is deposited in the paddocks. The estimated proportion of excreta deposited in these non-productive areas is 10–35% of the total (Nguyen and Goh, 1994).

The quantitative measurement of urine patch distribution is difficult, and as a result is not well understood (Moir et al., 2011). Recent research by Moir et al. (2006); Moir et al. (2011) and Dennis et al. (2011) developed new methodology for quantitatively measuring urine patch distribution in the field using a real-time kinematic global positioning system (RTK-GPS). Moir et al. (2011) reported mean annual urine patches on a dairy farm to be 6240 (±124) patches ha⁻¹. Dennis et al. (2011) reported 0.359 urine depositions per grazing hour, covering 14.1-20.7% of the soil surface annually. These values were driven mainly by stocking density.

### 2.4.3 Fate of urinary N

The fate of urine across multiple pathways can be determined by mass balance using ¹⁵N. The recovery of urinary ¹⁵N from leachate, pasture and N₂O fractions have been previously reported by Fraser et al. (1994), Clough et al. (1996), Clough et al. (1998b), Di et al. (2002), and Decau et al. (2003). On average, around 80% of the ¹⁵N in mass
balance studies is recovered, leaving around 20% ‘unaccounted for’ (Allison, 1955; Clough et al., 2001). To resolve this so-called $^{15}$N enigma, Clough et al. (2001) measured
the fate of $\text{NO}_3^{-}$-$^{15}$N applied to soil cores in a gas tight glovebox. This study determined
that nearly 23% of the $^{15}$N was recovered as N$_2$ and N$_2$O lost via convective gas transport
through the base of the soil cores, and as entrapped gas that was released upon destructive
soil sampling.

### 2.4.3.1 Pasture

Pasture growth usually visibly increases in response to urine patch deposition (Figure
2.7), mainly due to the added N and K (During and McNaught, 1961; Haynes and
Williams, 1993). This visible effect is usually present for about 3 months (Whitehead,
1995). Plant response to urine is greatest in spring and autumn (Dale, 1961), where
growth is less likely to be restricted by environmental conditions such as low soil
moisture (i.e. summer) or temperatures (i.e. winter). The growth response is dominated
by the grass component of the pasture, as clover is a poor competitor for the available N,
having a depressing effect on the clover content (Haynes, 1981). Ball et al. (1979)
showed 7 weeks following applications of 0, 300 and 600 kg ha$^{-1}$ of urinary N, the
proportion of clover in the total pasture yield was 48, 19, and 12%, respectively.

The deposition of urine can also ‘scorch’ the pasture. Urine scorch occurs when the high
N content and temporary rise in soil pH during urea hydrolysis causes ammonia toxicity,
which, coupled with the high salt content of the urine, has a detrimental effect on plant
roots (Richards and Wolton, 1975). Generally the higher the N concentration and ionic
strength of urine, the larger the extent of pasture scorching (Groenwold and Keuning,
1988). Recovery of pasture from urine scorch depends on the seasonal environmental
limitations, but can take up to 10 months (Dale, 1961; Deenen and Middelkoop, 1992). A
consequence of urine scorch, is the gradual ingress of weed species and the deterioration
of the sward quality (Keuning, 1980).

### 2.4.3.2 Microbial immobilisation

The response of soil microbial populations to urine depositions over time, is poorly
understood (Patra et al., 2005; Orwin et al., 2010). The constituents of urine can alter the
chemical and physical properties of the soil which, in turn, affects the soil microbial
populations (Haynes and Williams, 1993). Studies have shown soil microbial biomass to
increase, e.g. Lovell & Jarvis (1996b), decrease, e.g. Orwin et al. (2010) and remain
unchanged, e.g. Williams et al. (2000). Although urine deposition can enhance microbial activity, some studies have observed severe disturbance and stress in microbial communities. Urea hydrolysis to NH₃ following urine deposition results in a rapid rise in pH, which, in conjunction with the salt content of the urine, causes rapid changes to osmotic pressure (Petersen et al., 2004). Accumulation of toxic compounds such as NH₃ and NO₂⁻ following urine deposition can also induce disturbance and stress to existing microbial communities (Petersen et al., 2004; Orwin et al., 2010). Furthermore, Orwin et al. (2010) showed that urine deposition resulted in changes to the community structure of ammonia-oxidising and nitrite-reducing bacteria. On the other hand, a study by Pettersson & Bååth (2003) suggested that microbial communities can adapt over time to changes in soil pH, showing that a microbial population adapted to pH increases similar to that under a urine patch within 8 days.

2.4.3.3 Leaching of urinary N

The high N loading rate within urine patches exceeds the pasture’s immediate N demands. The residual NO₃⁻-N in the soil is therefore prone to gaseous loss and leaching, when drainage occurs. The amounts of NO₃⁻-N leached from cattle urine patches have been reported to range from 11 to >170 kg N ha⁻¹ (Hood, 1976; Silva et al., 1999; Di and Cameron, 2002b; Silva et al., 2005).

As described in Sections 2.5.1 and 2.5.2, leaching of urinary N is affected by the concentration of N constituents in the urine, which itself is affected by the diet and physical characteristics of the individual animal, depth to which the urine penetrates the soil, the frequency, volume and area covered by urine patches, as well as soil, environmental and climatic factors.

Seasonal variability occurs, with greater NO₃⁻-N leaching losses measured when urine is deposited in the autumn/winter months, when rainfall and soil moisture is high and plant uptake is low (Di and Cameron, 2002a, b; Decau et al., 2003; Cameron et al., 2013). When urine is deposited in the warmer, drier seasons, there is still the risk of residual N leaching in the following drainage season, however, additional plant uptake, immobilisation and denitrification in the intervening time may reduce the total amount lost.

Higher stocking rates increase leaching of urinary N due to greater numbers of urine patches per unit area of land. There is also greater potential for overlapping of urine
patches to occur under higher stocking rates. High urinary N loading can also occur under stock congregation areas (Sanderson et al., 2010). Congregation areas may include flat land, under trees, gateways, around water troughs and areas where hay or silage are fed out. Studies on hill country have shown that around 55% of urine deposition occurs on stock camps that account for 15-31% of the grazed area (Haynes and Williams, 1993).

2.4.3.4 Gaseous emission of urinary N

Emissions of NH₃ can be high following deposition of urine. The rapid hydrolysis of the N deposited in a urine patch results in the generation of OH⁻ and HCO₃⁻ ions and a temporary rise in soil pH. This coupled with the high soil NH₄⁺ concentrations creates ideal conditions for NH₃ production (Haynes and Sherlock, 1986). Peak soil NH₄⁺ concentrations following urine deposition occur within 24 h, and urea hydrolysis can be complete within 48 h (Ball et al., 1979; Sherlock and Goh, 1984; Lovell and Jarvis, 1996a; Zaman et al., 2008). Ammonia loss can represent from 4-46% of the applied N from a urine patch (Haynes and Williams, 1993; Cameron et al., 2013).

Urine deposition can also result in increased N₂O emissions. Reported N₂O-N emissions from urine patches generally range between 0.1-4.0% of the urine applied (de Klein et al., 2001). However, emissions of up to 14% have been reported from poorly drained clay soils in a laboratory study (Lovell and Jarvis, 1996a; de Klein et al., 2001). Emissions of N₂O from grazed pasture systems can be enhanced where soils are compacted from animal treading. Compaction decreases the soil pore volume, particularly the larger pores, decreasing aeration and thereby increasing the capacity for denitrification associated N₂O evolution (Oenema et al., 1997; Ball et al., 2012).

The use of nitrification inhibitors has been shown to significantly reduce N₂O emissions by up to 81% (Di et al., 2010) from urine patches (Di and Cameron, 2003; de Klein and Ledgard, 2005; Di and Cameron, 2006; Di et al., 2007; Di and Cameron, 2008; Luo et al., 2010; Monaghan et al., 2013). This is a result of directly reduced nitrification-associated N₂O emissions and/or a reduced NO₃⁻-N pool resulting in decreased denitrification-associated N₂O emissions (Oenema et al., 1997). The factors affecting N₂O loss from urine patches vary widely and include those described earlier in Section 2.2.9. Other factors can also include the extent of treading, urine composition and N content, soil type and environmental conditions at the time and after deposition (Oenema et al., 1997).
2.5 Nitrogen fertiliser in pastoral systems

The main function of fertiliser-N in the pastoral agriculture is to overcome environmentally driven N limitation, and enhance pastoral growth and thus feed supply for grazing animals (Parfitt et al., 2006; Dairy NZ, 2011). Over the last 2 decades there has been a near 6-fold increase in the amount of N applied as fertiliser in New Zealand; from 52,000 tonnes in 1990, to 333,000 tonnes in 2010 (Austin et al., 2006; Ministry for the Environment, 2012).

Until recently, the cost of N fertiliser has been low relative to the value of increased dry matter yields, which arguably led to a common mindset that applying more fertiliser N than was necessary provided a “low-cost insurance” against plant demand uncertainties (McIsaac, 2003). In New Zealand, the most commonly applied N fertiliser is granulated urea, which comprises 46% N and is conventionally applied over pasture by spreading urea prills (NZFMRA, 2007).

2.5.1 Fate of fertiliser N

Similarly to determining the fate of urine, the fate of fertiliser N applied to pastoral systems can be determined by $^{15}$N mass balance using $^{15}$N enriched fertiliser. The recovery of fertiliser $^{15}$N from various fractions has been previously reported by Dowdell and Webster (1980), Ledgard et al. (1988), Di et al. (1999), and Silva et al. (2005).

2.5.1.1 Pasture

The response of grass swards to different rates of N fertiliser application have been studied extensively, usually where fertiliser is applied in 3-7 even applications during the growing season (Whitehead, 1995). The results of these trials show that herbage yield increases considerably with increasing fertiliser application rate, up to approximately 300 kg N ha$^{-1}$, where at application rates above this, the herbage response per kilogram fertiliser applied declines, and the cost:production ratio of the fertiliser also declines considerably (Figure 2.9) (Whitehead, 1995).
Plant response to fertiliser N is also affected by the availability of other nutrients, temperature, soil moisture, and daylight hours. In other words, when plant growing conditions are favourable, the response of pasture yield to fertiliser N is greatest (Whitehead, 1995).

### 2.5.1.2 Microbial immobilisation

The application of fertiliser N results in increased activity of NH3 oxidising and nitrifying bacteria in the short term i.e. until the applied urea has been oxidised to NO$_3^-$, however, these bacteria represent only a small proportion of the total microbial biomass. The soil microbial population competes with plants for the available N (Kaye and Hart, 1997). Some studies show that following fertiliser application, microbial N uptake out-competes plant N uptake for the first few hours only (Hodge et al., 2000; Inselsbacher et al., 2010). After one day, the plants begin to outcompete the microbes for N because they can retain the N in their tissue for longer and take advantage of N fluxes from microbial turnover (Kaye and Hart, 1997; Inselsbacher et al., 2010).

The application of fertiliser can stimulate microbial activity and the rate of background soil organic N mineralisation. This is referred to as the ‘priming effect’, a term that was first introduced by Bingemann, (1953), and also generally results in increased microbial
N immobilisation. In studies of soils that received $^{15}\text{N}$ labelled fertiliser, a common observation was for enhanced pasture utilisation of ‘unlabelled N’ from the background soil N pool (Jenkinson et al., 1985; Kuzyakov et al., 2000).

Conversely, some studies suggest that N fertiliser application reduces microbial activity. A review by Treseder (2008) investigated the response of microbial biomass to fertiliser N additions, and found that on average, microbial biomass declined by 15% under N fertilisation, with declines being more obvious in longer term studies with greater N inputs.

### 2.5.1.3 Leaching

Nitrogen fertiliser is generally only leached if excessive fertiliser rates, above what the pasture can utilise, are applied. This being the case, any residual inorganic N is likely to be leached in the next drainage event. The amount of N leached increases with increasing fertiliser rate, as illustrated by Goulding (2000) in Figure 2.10 below. In another study, Hood (1976) reported leaching losses of 11 and 54 kg N ha$^{-1}$ yr$^{-1}$ from fertiliser N applications of 250 and 750 kg N ha$^{-1}$ yr$^{-1}$, respectively. In addition, the timing of fertiliser application can affect N leaching. If high rates of fertiliser are applied under saturated conditions, during drainage or when other environmental factors are limiting plant growth, the likelihood of fertiliser associated N leaching is much greater (Cameron and Haynes, 1986; Di and Cameron, 2002b). It should be noted that leaching of mineralised soil organic matter can occur when no fertiliser is applied, which can result in leaching losses of up to 10 kg N ha$^{-1}$ yr$^{-1}$ (Goulding, 2000; Di and Cameron, 2002b).

![Figure 2.10](image.png)

**Figure 2.10** Leached N from the Broadbalk Experiment at Rothamstead Experimental Station [Cameron et al. (2013) adapted from Goulding (2000)].
2.5.1.4 Gaseous N emissions

Emission of NH₃ from under urea fertiliser is generally not as high as from under urine because the N loading and thereby generation of OH⁻ ions via urea hydrolysis is much lower, however, it can still represent a considerable loss of N from the system ranging from 0-65% of the applied fertiliser N (Haynes and Williams, 1993; Cameron et al., 2013). Ammonia emissions following fertiliser application can be reduced by incorporating fertiliser into the soil, applying fertiliser prior to rainfall or irrigation (Black et al., 1987) or the use of urease inhibitors (Saggar et al., 2012).

The increased use of fertilisers has increased both direct emissions of N₂O from agricultural soils, and indirect emissions (e.g. denitrification of leached NO₃⁻-N) (de Klein et al., 2001). Nitrous oxide emissions from fertiliser generally constitute 0.1-2% of the N applied (de Klein et al., 2001) although some studies have measured higher emission factors e.g. 4.1% (Kaiser et al., 1998) and even up to 12% (Velthof et al., 1996).

Some studies suggest that different fertilisers result in greater N₂O emissions e.g. higher emissions were observed under wet conditions under NO₃⁻ based fertilisers compared to ammonium or urea fertilisers (Velthof et al., 1996; de Klein et al., 2001). However, other studies have observed N₂O emissions from urea to be equal or higher than NH₄⁺ and/or NO₃⁻ based fertilisers (Clayton et al., 1997).

Fertiliser associated N₂O emissions are highly variable by day, season and year, and are affected by a myriad of environmental factors including temperature, rainfall, soil moisture and oxygen status, soil organic C content, pH and windspeed; and also management practices such as fertiliser application rate and timing, tillage, and irrigation (Eichner, 1990).

2.6 Interaction of fertiliser N with urine patches

It has been suggested that fertiliser application over urine-affected areas (i.e. grazed pasture) leads to an increased risk of N leaching, N₂O emissions and decreased fertiliser N use efficiency (de Klein et al., 2001; Mackenzie et al., 2011; Cameron et al., 2013). However, few studies have quantitatively investigated the interaction between concurrent mineral N fertiliser application and urine patch deposition and the subsequent effect on N fate, particularly losses, from pastoral systems. Furthermore, no studies to date have attempted to differentiate the fertiliser associated N losses from the urinary N losses.
Decau et al. (2004) investigated N leaching as affected by $^{15}$N amended cattle urine, season of urine deposition and N fertiliser rate in combination and found that the greatest influence on N leaching loss was the amount of N deposited as urine. Decau et al. (2004) observed increased leached N concentrations under urine patches with increasing fertiliser application rate. However, decreased drainage with increased fertiliser rate was also observed, presumably due to increased plant growth and evapotranspiration, which impacted total leaching. Furthermore, Decau et al. (2004) calculated that the addition of 1 kg N as fertiliser (application rate in the range 0-30 g N m$^{-2}$), resulted in an additional 57 mg N leached; and the addition of 1 kg N as urine (in the range 0-16.5 g N m$^{-2}$) resulted in an additional 170 mg N leached.

In a lysimeter study with combined urine and fertiliser treatments, Silva et al. (1999) found that under urine (1000 kg N ha$^{-1}$), 12% of the urine N was leached, but when the urine application was combined with urea fertiliser (200 kg N ha$^{-1}$), the N leached increased to 14% of the total N applied. This supports the idea that fertiliser N plays a smaller role in N leaching compared to urine patches, but $^{15}$N enrichment was not used in either the urine or the fertiliser treatments, so the origin of the leached N cannot be confirmed. A further study by Silva et al. (2005) investigated the impact of urea fertiliser and effluent with and without urine on N losses and plant uptake, and also reported that the urine plus urea treatment resulted in the greatest leaching losses (Figure 2.11).

![Figure 2.11 Total NO$_3^-$-N leached from lysimeters (a) without urine and (b) with urine. (DE is dairy effluent) (Silva et al., 2005).](image-url)

Although $^{15}$N labelled urine was used in this experiment, it was only applied to the urine + dairy effluent and urine alone treatments, therefore, again the relative contribution of urine and/or fertiliser to the leached N pool was not quantified.
While some studies have investigated the fate of N under urine patches amended with fertiliser, (e.g. Decau et al. (2004), Silva et al. (2005), Leterme et al. (2003)), none have used $^{15}$N to partition the fertiliser contribution to N loss. Where urine patches and fertiliser are applied to pasture in conjunction, understanding of the relative partitioning of the fertiliser and urine associated N is important in increasing our understanding of the fate of N from pastoral systems. Quantifying fertiliser associated N loss from the urine patch–fertiliser interaction is of particular importance and has implications for the requirement of precision agriculture technology in New Zealand dairy farming systems.

2.6.1 Precision agriculture in New Zealand

In New Zealand dairy farming systems, N fertiliser is normally applied at a uniform rate by ground and/or air based broadcast spreaders (Murray et al., 2007). In a bid to increase the accuracy and evenness of fertiliser spreading in New Zealand, quality assurance programmes including the Fertmark Quality Assurance Programme (NZFQC, 2002) the Spreadmark Quality Assurance Programme (NZFQC, 2006), and the Code of Practice for Fertiliser Use in New Zealand (NZFMRA, 2007) have been developed. Precision agriculture is an all-encompassing term given to a range of technologies that allow the improved and more precise management of agricultural systems by recognising the large variation in the factors that limit production from agricultural land (Bramley, 2009). Current precision fertiliser application research and technology in New Zealand aims to optimise fertiliser use efficiency by matching the physiological requirements of plants within individual areas of a field or paddock to fertiliser inputs through the use of global positioning systems (GPS) and variable rate technology (Lawrence, 2007).

A recent development by Agri Optics NZ Ltd is the Smart-N™ fertiliser application system (still under development), which is designed to selectively apply liquid fertiliser to pasture that avoids urine and dung patches (Yule and McVeagh, 2011). The Smart-N™ system uses Weedseeker® optical sensor technology to detect urine and dung patches in the field (Mackenzie et al., 2011). The Weedseeker® technology consists of a highly sensitive optical sensor that distinguishes between high and low NDVI (Normalised Difference Vegetation Index) and was originally designed to detect weeds for activation of herbicide application (Mackenzie et al., 2011). Adaptation of this technology aims to identify urine patches by detecting the difference in reflectance of pasture affected by a urine patch, and pasture that is unaffected. Following this, spray technology will deliver
(or halt delivery of) the fertiliser product to the identified area (Mackenzie et al., 2011). The system is also capable of delivering a nitrification inhibitor to the identified urine patches.

Because there is a dearth of information surrounding the interaction of urine patches and fertiliser, and the fate of the fertiliser associated N, little can be known about how precision fertiliser application technology affects N losses from dairy farming systems, therefore further research is required to determine the environmental implications of this technology.

2.7 Process based modelling in science

Models are mathematical representations of our understanding of the mechanisms that govern natural processes (Tedeschi, 2006). There are a wide range of environmental models available for a diverse range of purposes, most of which fall into three categories: explanation, prediction and decision support (Johnson, 2001). Models are increasingly useful in environmental/agricultural science. They can provide decision support, offer predictive information where there is limited capability for empirical measurement; they can aid with experimental design and hypothesis development and discriminate between mechanisms to answer specific questions. They also offer a lower cost alternative (or additive) to large experimental campaigns and can integrate various components of a system, giving a holistic view of its behaviour (Thornley and Johnson, 2000).

In New Zealand, information from agricultural models is increasingly relied upon to inform and support farm management decisions, consulting advice and policy-making decisions, therefore, it is important that the model users, and those subsequently affected are not only confident in its performance, but also aware of its limitations and uncertainties. The increasing use of models and/or modelled outputs by managers, consultants, farmers, (i.e. non-modellers) increases the risk of models being used for different purposes than were originally intended and the resulting outputs and/or predictions being misinterpreted. For this reason ‘good practice’ and quality control measures have been suggested by researchers e.g. Risbey et al. (1996), Jakeman et al. (2006), and Tedeschi, (2006), to reduce these risks and increase awareness of the modelling process, the potential limitations, how results should be viewed, and questions that should be asked.
The modelling process involves several key steps of which the first and most important is to define a clear objective of the model. The form and content of a model depends entirely on its intended purpose (Johnson, 2001). Jakeman et al. (2006) outlined 10 incremental steps that are crucial for successful model development and application. It is intended that these steps are revisited or reassessed numerous times during model development (Figure 2.12).

![Diagram of the modelling process](image)

Figure 2.12 Ten iterative steps for model development (Jakeman et al., 2006).

### 2.7.1 Types of models

Mathematical models can be separated into a number of categories. To name a few, there are deterministic models, which make unique, definitive predictions without reference to any probability distribution; stochastic models, where predictions are calculated using probability distributions and randomness (Johnson, 2011); dynamic models, which predict how a system responds with the passage of time, and static models which do not account for a time variable. Most agricultural models are dynamic process-based models,
therefore we are concerned with these types of models in this review. The most commonly used dynamic models include empirical, mechanistic, teleonomic.

- **Empirical models**

Empirical models are fundamentally mathematical descriptions of observed data. Empirical models describe observed behaviour within a single hierarchical level, in terms of the attributes of that level alone, without concern for biological or physical theory. Empirical models do not present any new information; rather they re-represent observed data. Empirical models are effective in summarising data and relationships and can provide practical tools for decision making. However, they do not give any indication of the factors or mechanisms that give rise to a given response, nor to the possible reasons behind a response (Thornley and Johnson, 2000).

- **Mechanistic models**

Mechanistic modelling constructs an explanation of behaviour at a certain hierarchical level which includes information on the underlying processes or ‘mechanisms’ behind that particular behaviour. Mechanistic models are generally more complex than empirical models at a given hierarchical level and do not tend to fit observed data as well as empirical models, a limitation caused by the fact that there are usually many more assumptions built into them. On the other hand, the content of mechanistic models is more comprehensive and applies to a greater range of systems and processes, with the ability to interrelate them (Thornley and Johnson, 2000).

- **Teleonomic models**

These are sometimes called “goal seeking” models and are constructed specifically for goal or purpose related behaviour. An example of teleonomic or goal oriented modelling could be the root to shoot partitioning in plants, where new plant tissue material is allocated to the roots and shoots in such a way that achieves the optimal growth rate in a particular environment (Thornley and Johnson, 2000). The “goal” or “purpose” is optimal plant growth and the calculation of the proportion of new tissue that is directed at the roots and/or shoots can be achieved by a teleonomic partitioning model.
2.7.2 Hierarchy and scale

In all biological systems, a series of hierarchical organisational levels exist, e.g. the typical hierarchical levels for plant science are illustrated below (Table 2.4).

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>Crop</td>
</tr>
<tr>
<td>(b)</td>
<td>Plant</td>
</tr>
<tr>
<td>(c)</td>
<td>Organs</td>
</tr>
<tr>
<td>(d)</td>
<td>Tissues</td>
</tr>
<tr>
<td>(e)</td>
<td>Cells</td>
</tr>
<tr>
<td>(f)</td>
<td>Organelles</td>
</tr>
<tr>
<td>(g)</td>
<td>Macromolecules</td>
</tr>
<tr>
<td>(h)</td>
<td>Molecules and atoms</td>
</tr>
</tbody>
</table>

Table 2.4 Hierarchical levels in plant science (Thornley and Johnson, 2000).

Each hierarchical level is an integration of the properties of the levels below it (termed scientific reductionism). Spatial and temporal scales typically become smaller at lower levels, with increasingly smaller sizes and faster processes (Thornley and Johnson, 2000). In modelling, the successful function at a particular level requires that the levels below it operate successfully; however, the opposite does not apply. The higher levels provide limitations, boundary values and driving functions (e.g. inputs and outputs) to the lower levels (Thornley and Johnson, 2000).

Scale is an important consideration in model development, and many models are designed at the plot or field scale, while end users, e.g. policymakers, often require information at regional and national scales (Addiscott, 2003). Ideally, models need to be applicable at all scales, and models with parameters that have the same significance at all scales should be applicable at all scales, e.g. soil volumetric water content, which has the same meaning and value for 1 cm³ of soil as it does for 1 km³ (Dumanski et al., 1998). When referring to land units, the notion of hierarchy is often associated with scale (i.e. plots lie within paddocks, which lie within farms, which lie within catchments, and so the hierarchy continues through regional, national and continental or global scales) (Addiscott, 2003).

2.7.3 Evaluation of models

The evaluation of model performance is a key step in model development indicating the level of precision, accuracy and confidence of model predictions (Tedeschi, 2006). There
are a range of methods used for performance evaluation of models including numerical, graphical and qualitative methods (Bennett et al., 2013). The evaluation of models has elements of subjectivity and they can be evaluated on a range of characteristics, however, a strong weighting should be given to the appropriateness of a model in relation to its objectives (Thornley and Johnson, 2000). Bennet et al. (2013) presents a comprehensive review of model evaluation performance methods, however, only the quantitative evaluation of modelled outputs against experimental or observed data will be discussed here.

2.7.3.1 Verification and validation

Verification and validation are words that are sometimes used interchangeably, however, in terms of modelling evaluation, they have very different meanings. Model verification refers to the process of ensuring a model performs as it was intended (in line with its original objectives), that the mathematical code is correct and that it accurately translates the biological assumptions behind the modelled process (Frey and Patil, 2002; Tedeschi, 2006).

Validation refers to the extent to which a model is rational in its representation of the system being modelled (Tedeschi, 2006). A common way of achieving this is evaluating the accuracy of the predicted outputs simulated by a model against observational data from scientific experiments (that was not used in the development of the model) (Bennett et al., 2013). It is important to note that although agricultural/environmental models represent a simplification of reality (Thornley and Johnson, 2000); so too do field or laboratory based experiments, albeit to a lesser degree. Furthermore, due to the error associated with both modelled and experimental data, an exact fit between the two is highly unlikely and undesirable. The goodness of fit is more an indicative measure of whether the model represents our understanding of the system.

The terminology of both verification and validation is often criticised (Oreskes et al., 1994) because it is physically impossible to prove that all or any components of a model are a correct representation of a real system. Furthermore, some models predict outcomes or relationships that simply cannot be measured experimentally and it is argued that they therefore, cannot be validated (Tedeschi, 2006). Popper (1992) suggested that “we can never justify theory, but we can justify our preference for a theory” which implies that
although we cannot verify/validate a model to prove it entirely accurate, we can discriminate between models based on our objectives.

2.7.3.2 Sensitivity analysis

Sensitivity analysis is another method used in determining the level of confidence in model performance. Sensitivity analysis is the systematic testing of how changes in the parameter values in a model affect the output results (Johnson, 2001). It can also identify parameters responsible for uncertainty in the model (Saltelli and Annoni, 2010). Parameters that result in significant changes to the outputs are particularly important and should be estimated as accurately as possible (Johnson, 2001). Sensitivity analysis is commonly carried out on one parameter at a time; however, it is important that the interactive effects on all outputs are determined. Alternatively more complex multi-parameter sensitivity analyses are often used.

2.7.4 Limitations of modelling

- Scale

Scale is an important concept in modelling, and the term ‘scale’ itself can be easily misinterpreted because it can refer to both large and small areas of land, for example, a large scale development refers to a large land area, whereas, a large scale map refers to a small land area (Addiscott, 2003). Also, government organisations and policymakers require information from models at national or regional scales, whereas many models are developed at a plot or paddock scale. Up-scaling of models reduces the accuracy of inputs and increases heterogeneity and down-scaling requires increased accuracy (Addiscott, 2003). Validation and error propagation are also potential issues arising from up or down scaling models (Addiscott, 2003).

- Decoherence

Decoherence is also associated with scale and is a term used to explain the loss of indeterminacy that occurs as smaller systems are amassed to make up larger ones. An analogy for this is the nature of physics at large and small scales. Historically, physics was based on large scale observations (e.g. Newton’s Law), however more recent quantum physics is associated with very small scales and is largely indeterminate (Addiscott, 2003). Relating this to agriculture, processes occurring at a large paddock or catchment scale should be more determinate than the same process at a small soil particle.
scale, e.g. considerable N$_2$O emissions from soil occur from randomly located microsites, where conditions are favourable for denitrification (Parkin, 1987). The exact location and the processes occurring in these sites are unpredictable, and very difficult to model at the plot or field scale. However, the establishment of predictive models for denitrification have been easier at the larger catchment scale (Groffman and Tiedje, 1989; Corre et al., 1996) due to the fact the soil moisture (which has a significant effect on denitrification) can be estimated based on topographical features in the landscape. This information, combined with other factors, such as soil and climate, can then be used to create denitrification models (Addiscott, 2003).

- **Error**

As described earlier, models cannot conceptually be 100% accurate. In a modelling context, error refers to the disparity between the modelled representation of a system, and our scientific understanding of the reality of the system (Heuvelink, 1998). There are three primary sources of model error:

(a) Input error: Model parameters such as soil properties and weather and/or climatic data always contain a degree of error. Some of these may be “human error” or mistakes, and although it is important to minimise this sort of error, little can be done to avoid it. What is of greater concern is statistical error which arises from either natural variation, or error introduced from measurements or estimation (Addiscott, 2003);

(b) Model error: A fault in the model itself can arise from “concept error”, i.e. an error in understanding, or deliberate simplification by the modeller of the system being modelled. There is no diagnostic test for this kind of error, however they may be exposed by sensitivity analysis and review critique, and should certainly be uncovered if the model is validated against observed data (Addiscott, 2003). Corrective action against model error is to simply change the model. As noted earlier, changes in scale may also require changes to a model (Addiscott, 2003). Another possibility is “error in translation”, where error occurs during the process of converting the concept or theory into a set of mathematical equations and computer code. Translation errors are usually revealed during model verification, and remediation will depend on the error (Addiscott, 2003);

(c) Output error: This can be a result of input error, model error or both. The relation between input and output error (in terms of variation) is the essence of error propagation.
The majority of variables measured for use as parameters in models have a certain amount of error, as do parameters that are inferred rather than measured (Addiscott, 2003). If a given model is non-linear, the error in the input contributes to the value of the mean of the output, and can significantly increase the output error. Error in multiple parameters or equations can also cancel each other out.

- Units and conversion factors

The choice of units can often cause problems and confusion in modelling. The mixture of units for mass (e.g. milligrams and kilograms), or area (e.g. square kilometres and hectares) within one model increases the potential for error and confusion. It is therefore important to have a consistent set of units throughout a model. If unit conversions are deemed necessary, these should be undertaken outside of the model, leaving it independent of conversion factors. Many modellers use the universally accepted International System of Units (SI) (Royal Society, 1975) where the basic units for mass, length and time are kilograms, metres and seconds respectively.

2.8 Summary

Increases in the industrial scale production of N fertilisers for agriculture has allowed for increases in the intensification and profitability of this sector. However, the loss of reactive N from agricultural systems to the wider environment, namely via leaching and gaseous emissions, contributes to some large present day environmental problems, as well as representing a waste and loss of potential productivity.

In pastoral systems, urine patches are considered the primary source of N loss, however, this review identified there is little quantification of the area actually affected by a urine patch (the ‘effective area’). This is important in determining how urinary N is partitioned between plant uptake and losses, and has important implications for the ability of predictive models and nutrient budgeting software to accurately estimate N losses.

In addition to this, the application of fertiliser over grazed pasture is an added contributing factor to N losses from urine patches; however, very little is known about the fate and dynamics of N loss when the two are applied concurrently. Also, while some research has quantified the fate of urinary N, in the presence of fertiliser, little attention has been paid to quantifying the fate of the fertiliser component, rather, it has been assumed to simply have an additive effect on N loss. Much research and development
into precision fertiliser technology has occurred (in particular urine/dung patch avoidance technology) without any quantification of its environmental implications for N loss reduction, making this an important research gap.

Finally, process-based models to aid in decision support and predictive estimates of N loss from pastoral systems are increasingly useful tools for farmers, councils and consultants; however, continued evaluation and validation of modelled outputs with experimental data can be an essential element of quality control. The employment of models to aid in policy making and farm management decisions will likely continue to increase with increasing reliance. While the aim of many of these decisions/policies is to improve environmental health, they also have the potential to have widespread social and economic impacts on individuals, communities and the pastoral agricultural sector in NZ as a whole. Therefore, it is important that models replicating pastoral systems are evaluated and validated against relevant experimental data where possible.

The review of literature has identified some key knowledge gaps. These include:

- A lack of understanding of the fate of fertiliser N when it is deposited on a urine patch;

- Quantification of the ‘effective area’ of a urine patch; and

- An ongoing requirement for the accurate prediction of the fate of N in pastoral systems using models, to (a) increase confidence in their use as decision support mechanisms, and (b) add value to and/or extrapolate on experimental data to answer scientific questions and increase understanding of the whole system behaviour.

The following research chapters address these knowledge gaps.
Chapter 3
Urine patch and fertiliser N interaction: effects of fertiliser rate and urine timing on the fate of N

3.1 Introduction

It is well recognised that the deposition of animal urine patches and the application of urea fertiliser in dairy farming systems contributes to N losses from the soil-plant system (Cameron et al., 2013). These losses include gaseous emissions (NH₃ volatilisation, N₂O and N₂ emissions) and the leaching of NO₃⁻-N and dissolved organic forms of N through the soil profile. It is well documented that urine-N dominates leaching losses in grazed dairy pastures because the urine-N rate far exceeds the ability of the affected pasture to utilise it, and the excess N remains in the soil until drainage and, ultimately, leaching occurs. The timing of urine deposition can also affect the extent of N loss, with the greatest risk of leaching loss being late autumn, winter and early spring, when temperatures are cooler, plant N uptake is low, and soil drainage occurs due to rainfall exceeding evapotranspiration (Wild and Cameron, 1980). Losses as a result of fertiliser N application can be minimal, if applied at rates that match plant demand and at times of low N leaching risk (Cameron et al., 2013), however, if fertiliser application is excessive, N use efficiency can be low, with the potential for residual N to be leached.

Despite numerous studies identifying the contribution of urine patches and fertiliser N use to N losses from the soil/plant system in dairy operations, few have studied the interaction of urine patches and fertiliser N and the effect such an interaction may have on N losses. Research and investment into precision fertiliser application has been undertaken in recent years with the development of variable rate application technologies, which can selectively apply liquid fertiliser to pasture avoiding urine and dung patches (e.g. Yule and McVeagh, 2011). Although the continued development of this technology will contribute to higher fertiliser-N use efficiency in dairy farming systems, there is limited knowledge surrounding the interaction of urine patches and fertiliser, and the fate of the fertiliser-N, suggesting there is also limited quantitative knowledge about the environmental implications of urine patch avoidance fertiliser application technologies and their effects on pastoral N losses.
The primary objective of this chapter was therefore to understand the interaction of urea fertiliser and urine on N fate where fertiliser is applied (following standard practice) to a urine patch deposited in either autumn or spring, and determine to what extent, if any, urea fertiliser enhances N losses from a urine patch. In this context, the “fate” of N refers to N leached, N2O emissions, pasture N uptake, and N retained in the soil. This study aimed to characterise the parameters indicative of N leaching losses (drainage, NO3⁻-N concentration of leachate, total inorganic N leached, total dissolved organic N leached) and gaseous N2O emissions as affected by the season of cattle urine deposition and the urea fertiliser regime. The key hypotheses tested were as follows:

a) An interaction will exist between fertiliser and urine treatments whereby greater fertiliser-N losses (leaching and N2O emissions) will be observed when fertiliser is applied over a deposited urine patch; and

b) these fertiliser-N losses will be greater under autumn deposited urine than spring deposited urine.

3.2 Methods and Materials

3.2.1 Lysimeter Collection

Thirty six large, intact soil monolith lysimeters (500 mm diameter by 700 mm depth) were collected on 14 December 2010 from the AgResearch No. 1 Dairy Farm, at the Ruakura Research Centre, Hamilton, New Zealand (latitude 37.779 °S, longitude 175.315 °E). The soil at the collection site was a Horotiu silt loam (Typic Orthic Allophanic Soil) (Hewitt, 1998). This soil is characterised by a dark brown, moderately weak, moderately developed medium nut structure silt loam A horizon (0 – 200 mm); a yellowish brown, moderately weak, moderately developed silt loam (200-400 mm), progressing to a sandy loam B horizon (200-600 mm); underlain by a yellowish brown loose sand or gravelly sand C horizon (Singleton, 1991). Horotiu soils are moderately permeable and very well drained. They have a potential rooting depth of up to 80 cm, the topsoil is structurally stable and available water in the root zone is generally high (Singleton, 1991). The soil was under ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.) pasture and had a history of regular grazing and fertiliser application. A total of 15 soil cores (7.5 cm depth) were taken randomly from the lysimeter collection site, bulked and analysed for basic soil chemical properties by NZ Labs (Table 3.1).
Table 3.1 Soil properties from lysimeter collection site

<table>
<thead>
<tr>
<th>Soil Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.9</td>
</tr>
<tr>
<td>Total C (g kg(^{-1}))</td>
<td>55</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}))</td>
<td>6.7</td>
</tr>
<tr>
<td>Olsen P (mg kg(^{-1}))</td>
<td>30</td>
</tr>
<tr>
<td>Sulphate sulphur (mg kg(^{-1}))</td>
<td>6</td>
</tr>
<tr>
<td>Potassium (cmolc kg(^{-1}))</td>
<td>0.65</td>
</tr>
<tr>
<td>Calcium (cmolc kg(^{-1}))</td>
<td>8.1</td>
</tr>
<tr>
<td>Magnesium (cmolc kg(^{-1}))</td>
<td>1.57</td>
</tr>
<tr>
<td>Sodium (cmolc kg(^{-1}))</td>
<td>0.14</td>
</tr>
<tr>
<td>Cation exchange capacity (cmolc kg(^{-1}))</td>
<td>23</td>
</tr>
<tr>
<td>Total base saturation (%)</td>
<td>45</td>
</tr>
</tbody>
</table>

The lysimeter collection procedure followed that described by (Cameron et al., 1992). The lysimeter casings were originally manufactured from steel plates (5 mm thick) that had been rolled and welded to make cylindrical casings 500 mm wide by 700 mm high. A steel internal cutting ring was welded to the inside of one end of the cylinder and extended 10 mm beyond the lower edge of the lysimeter (Figure 3.1a and b). Lysimeter casings were arranged within a marked area 1.5 m wide by 30 m long. A 1.0 m wide by 1.5 m deep trench was dug outside the marked area by a hydraulically operated digger to aid manual digging. The turf around the internal edge of the lysimeter casings was cut with a sharp knife and the turf and soil around the outer edge of the casing was dug away. The casing was then carefully pushed over the exposed monolith directly below the cutting edge, with the procedure repeated until the lysimeter casing was completely filled with the soil monolith. As a result of the internal cutting ring, there was an annular gap between the soil monolith and the casing. To prevent damage to the soil monolith, these gaps were packed with 5 mm thick lengths of wood.

The lysimeters were gently lowered onto their side to attach the base plates. The soil at the base of the lysimeter was levelled with the base of the casing. The base plates were attached by four steel rods with circular lugs (Figure 3.1d) evenly positioned around the lysimeter through lifting flanges (Figure 3.1c). The lysimeters were then removed from the trench by attaching a chain to the lugs and lifted out with the digger.
The lysimeters were then brushed down to remove loose soil, and the base plate was sealed to the lysimeter casing by applying silicone RTV sealant (Dow Corning Silastic ® 1080), which, upon drying, was covered with strong polyurethane adhesive (Holdfast Gorilla Grip Express). Meanwhile vasoline (petrolatom) was liquefied in large 20 L tin drums by heating on gas-fired hotplates. Once the entire drum was liquefied, it was left to cool for approximately thirty minutes. This slightly thickened the consistency of the melted vasoline so that upon contact with the soil, it set immediately, rather than being absorbed into the soil pore spaces. The 5 mm packing lengths between the soil monolith and lysimeter casing were removed and the liquified vasoline was administered through a
funnel into the annular gap between the soil monolith and the lysimeter casing. The vasoline was observed to flow freely from the delivery point, around the entire annular gap, to completely surround the outside of the soil monolith. The vasoline cooled on contact with the soil and the lysimeter casing, which later solidified to form a water-tight seal between the monolith and the casing, preventing preferential flow between the soil and the casing.

The lysimeters were transferred from the collection site using a small crane (Hiab) on the back of a truck, to an empty lysimeter trench approximately 1 km from the collection site. Drainage outlets were screwed onto the bottom of the base plates, and the lysimeters were positioned on both sides of the trench (18 on each side). Each side of the lysimeter trench had a series of 0.8 m lengths of 150 mm diameter pipes, underlain by gravel. The exposed surface of each piece of pipe had a circular 20 mm diameter hole, which the drainage outlet on the base of the lysimeters protruded through (Figure 3.3). The area between each length of pipe was filled in with sand, to create a relatively level surface for the lysimeter installation. Once the lysimeters were in place, they were accurately levelled, and the trench was back-filled with soil. The pasture at the collection site was suffering from drought so each lysimeter received 25 kg N ha⁻¹ urea fertiliser with 10 mm of water. The lysimeter collection site was back-filled, levelled and re-sown with pasture.

### 3.2.2 Bulk Density

Dry soil bulk density was determined for five depths at the lysimeter collection site (0-10, 10-20, 20-40, 40-60, 60-70 cm). Cylindrical soil core samplers (6 cm deep, 10 cm diameter) were used to collect undisturbed soil samples in duplicate at each depth. These soil samples were weighed, oven-dried for 24 hours at 105°C, then re-weighed, and the bulk density calculated as detailed in Equation 3.1 below.

\[
P_b = \frac{M_s}{V} \quad \text{(3.1)}
\]

Where \(P_b\) = dry bulk density (g cm⁻³); \(M_s\) = mass of oven dry soil (g), and \(V\) = volume of soil core (cm³).
Figure 3.2 (a) to (g) Lysimeter collection process
3.2.3 Experimental design and treatments

The experimental design consisted of three urine treatments (nil, autumn and spring applied urine) and three N fertiliser rates (0, 200 and 400 kg N ha\(^{-1}\) yr\(^{-1}\)) (Table 3.2). The treatments were arranged in a randomised complete block design with 4 replicates per treatment, a total of 36 lysimeters.

Table 3.2 Lysimeter treatments

<table>
<thead>
<tr>
<th>Name</th>
<th>Treatment</th>
<th>(^{15})N fertiliser rate (kg N ha(^{-1})yr(^{-1}))</th>
<th>Urine (kg N ha(^{-1})yr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F0U0 Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>F2U0 (^{15})N Urea 200</td>
<td>200 (25 kg N ha(^{-1})x 8)</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>F4U0 (^{15})N Urea 400</td>
<td>400 (50 kg N ha(^{-1})x 8)</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>F0UA Urine (Autumn)</td>
<td>0</td>
<td>800</td>
</tr>
<tr>
<td>5.</td>
<td>F2UA (^{15})N Urea 200 + Urine (Autumn)</td>
<td>200 (25 kg N ha(^{-1})x 8)</td>
<td>800</td>
</tr>
<tr>
<td>6.</td>
<td>F4UA (^{15})N Urea 400 + Urine (Autumn)</td>
<td>400 (50 kg N ha(^{-1})x 8)</td>
<td>800</td>
</tr>
<tr>
<td>7.</td>
<td>F0US Urine (Spring)</td>
<td>0</td>
<td>800</td>
</tr>
<tr>
<td>8.</td>
<td>F2US (^{15})N Urea 200 + Urine (Spring)</td>
<td>200 (25 kg N ha(^{-1})x 8)</td>
<td>800</td>
</tr>
<tr>
<td>9.</td>
<td>F4US (^{15})N Urea 400 + Urine (Spring)</td>
<td>400 (50 kg N ha(^{-1})x 8)</td>
<td>800</td>
</tr>
</tbody>
</table>

Prior to treatment application, the lysimeters each received 800 mm of water (> 1 pore volume) over a period of 10 days to flush any antecedent NO\(_3\)-N from the lysimeters. The experiment began on 21 February 2011 and continued until 28 August 2012. The lysimeters received treatments of \(^{15}\)N enriched (5 atom%) urea fertiliser at three rates (0, 200 and 400 kg N ha\(^{-1}\)), applied as 8 annual, evenly split, applications (representing ‘best practise’), of 0, 25 and 50 kg N ha\(^{-1}\), respectively, with and without either a single autumn urine or spring urine application. Fertiliser was applied evenly in a powder form over the whole area of the lysimeter on the following dates: 21 Feb 2011; 28 Mar 2011; 4 May 2011; 31 May 2011; 31 Aug 2011; 29 Sep 2011; 7 Nov 2011; 5 Dec 2011; 28 Feb 2012; 30 Mar 2012; 30 Apr 2012; 31 May 2012. The fertiliser was washed in with 10 mm of water to reduce NH\(_3\) volatilisation (Black et al., 1987). The 200 kg N ha\(^{-1}\) yr\(^{-1}\) fertiliser rate represented a ‘best practise’ annual fertiliser rate for a dairy system in the Waikato region, while the 400 kg N ha\(^{-1}\)yr\(^{-1}\) rate represented a more intensive dairy system.

A total of 1.8 L of cow urine was applied to the relevant lysimeters on 4\(^{th}\) May 2011 (autumn) and 31 Aug 2011 (spring). Fresh dairy cow urine was collected from the
AgResearch dairy farm at Tokanui and standardised to a concentration of 8.0 g N L⁻¹ by diluting with deionised water. The urine concentration and volume were selected based on an estimated average New Zealand pasture based dairy cow urination event. Urine was applied at a rate of 10 L m⁻² over the entire area of the lysimeter, giving an equivalent application rate of 800 kg N ha⁻¹. The urine was then washed in with 10 mm of water to (a) reduce NH₃ volatilisation and (b) to prevent urine scorch. When water was applied to wash in the fertiliser and/or urine, the same amount of water (10 mm) was applied to all lysimeters, including controls. The treatments and their application timing plan are outlined below in Table 3.3.

<table>
<thead>
<tr>
<th>Application time (end of month)</th>
<th>¹⁵N fertiliser (25 or 50 kg N ha⁻¹)</th>
<th>Urine (800 kg N ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>March 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>April 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>May 2011</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>June 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 2011</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>September 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>October 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>November 2011</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>December 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>February 2012</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>March 2012</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>April 2012</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>May 2012</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

3.2.4 Leachate collection and analysis

After installation of the lysimeters, a length of plastic tubing was attached to the lysimeter drainage outlet, which extended to a plastic leachate collection container (20 L capacity). Each collection container was housed inside a box that was below the level of the drainage outlet, to ensure that drainage water would flow into the collection container and not stagnate inside the tubing (Figure 3.3).
Figure 3.3 Individual lysimeter setup.

The collection containers were checked following each rainfall event, and leachate was collected when the containers were approximately half full, or once the rainfall stopped. The volume of leachate was measured by recording the weight of the leachate-filled containers using portable weighing scales, then subtracting the tare weight of the container. A 250 mL sample was collected for analysis and the remainder was discarded. Leachate was analysed for NO₂⁻-N + NO₃⁻-N and NH₄⁺-N, using a Skalar SAN++ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). The NO₃⁻-N analysis involved cadmium reduction to NO₂⁻ followed by diazotisation with sulphanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form an azo dye measured colourimetrically at 540 nm. The NH₄⁺-N method is based on the modified Berthalot reaction where NH₃ is chlorinated to monochloramine which then reacts with salicylate and this is then oxidised to form a blue/green coloured complex.
which is measured colourimetrically at 660 nm. Dissolved organic N, and total organic C were analysed using a Shimadzu TOC-VCSH/TNM-1 analyser. Total C is determined by the “oxidative combustion-infrared” where CO₂ is measured through infrared detection after injection into dilute phosphoric acid. The difference between total C and inorganic C was organic C. Total N was determined by “oxidative combustion-chemiluminescence”. Organic N was calculated as the difference between total N and inorganic N.

3.2.4.1 Leachate ¹⁵N diffusion

The leachate samples were also analysed for NH₄⁺-¹⁵N and NO₃⁻-¹⁵N using EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry). The samples were prepared for analysis using the diffusion methodology described by Brooks et al. (1989). In summary, 7 mm diameter discs of Whatman GF/D filter paper were pierced with 80 mm lengths of stainless steel wire. These were then spring loaded across the inside ledge of 250 mL specimen container lids (LabServ® LB32250) and 10 µL of 2.5 M KHSO₄ was pipetted onto the filter discs. These were set aside for no longer than 10 minutes to minimise any potential air contamination. The equivalent of 50 µL of N, or 0.2–80 mL aliquots of leachate, (the volume depending on the NH₄⁺-N/NO₃⁻-N concentration of the solution) were placed in the 250 mL specimen containers along with a 4 mm glass bead. For NH₄⁺-¹⁵N analysis, approximately 0.2 g of furnace-dried MgO, was added, to raise the pH of the solution, and then the lid (with the spring loaded acidified filter disc) was quickly closed. The container was then carefully but thoroughly mixed, creating a cloud of suspended MgO. Mixing was important to ensure complete reduction of NO₃⁻-N to NH₄⁺-N, but care was taken to prevent the basic leachate solution from contacting the acidified filter
paper and neutralising the acid. For NO₃⁻¹⁵N analysis, the same method was applied, a 0.2 g scoop of MgO was added to the leachate aliquot, however, this time the specimen containers were left uncovered overnight, to allow any NH₄⁺-N in the sample to volatilise. The following day, the filter paper discs were acidified, and 0.4 g of Devarda’s alloy was added, before closing the lid, and mixing the solution as described above. The containers were left at room temperature (≈20˚C) for 6 days without further mixing.

The filter paper discs were removed at the end of 6 days and dried in an oven at 35˚C. The filter papers were then carefully placed into tin capsules, which were tightly folded and placed in an auto-analyser tray. These were analysed for ¹⁵N atom % by direct combustion using an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20). A detailed description of ¹⁵N analysis by IRMS is given in Section 3.2.8.

The ¹⁵N recovered as NH₄⁺-¹⁵N and NO₃⁻¹⁵N in the leachate as a percentage of the total ¹⁵N fertiliser applied was calculated using Equation 3.2 (Cabrera and Kissel, 1989):

\[
¹⁵N_{Recovered} = \frac{100}{\text{f}} \times \frac{p \times (c - b)}{f \times (a - b)}
\]  

Where \(p\) = the moles of NH₄⁺-N or NO₃⁻-N in the sample; \(f\) = the moles of N in the ¹⁵N enriched fertiliser applied; \(c\) = atom % of the sample; \(a\) = atom % of the ¹⁵N enriched fertiliser applied (5.0 atom %); and \(b\) = atom % ¹⁵N abundance of the control (0.3663).

### 3.2.5 Nitrous oxide collection and analysis

The lysimeters were affixed with stainless steel gas rings. They were fixed to the top of the lysimeter casing using silicone sealant. The gas rings consisted of a water trough, to create an air-tight seal when the headspace chambers were applied. The headspace chambers (internal volume 0.0176 m³) were each equipped with a three way stopcock attached to a length of rubber tubing, to facilitate headspace gas sampling, and a sealable vent, to ensure air pressure inside the headspace was equilibrated with atmospheric pressure. The chambers were insulated with a thin layer of styrofoam that was attached and sealed with duct tape.
3.2.5.1 \textit{N}_2\textit{O} Sampling procedure

A 50 mL sample was drawn into a 50 mL gas tight syringe, and pushed back into the headspace three times to mix the gas inside the chamber. A 10 mL sample was then collected and injected into a previously evacuated 6 mL glass vial (over-pressurising it) fitted with a screw cap and rubber septum (Exetainer\textsuperscript{®}; Labco Ltd, High Wycombe, UK). Sampling was executed at time 0, 30 and 60 minutes. Ambient air samples were also collected at these times. Nitrous oxide measurements were undertaken twice per week, for the first two weeks following any fertiliser and/or urine treatment applications, and once per week thereafter. The first N\textsubscript{2}O measurement was taken on Day 3 (23 Feb 2011) and the final measurement taken on Day 373 (28 Feb 2012) with a total of 59 measurements over the course of the experiment.

In addition to this, N\textsubscript{2}O samples were collected for $^{15}$N analysis using the same technique described above, except, sampling was executed after 180 minutes (3 h), and a 20 mL sample was collected and injected into a previously evacuated 12 mL vial (Exetainer; Labco Ltd, High Wycombe, UK). Measurements for N\textsubscript{2}O-$^{15}$N were taken less frequently, once per week for the first two weeks following fertiliser and/or urine application, and once every two weeks thereafter. Samples for $^{15}$N\textsubscript{2}O were only collected from lysimeters receiving $^{15}$N fertiliser treatments, as well as two controls receiving no fertiliser or urine (lysimeters #13 and #27).
The soil surface pH of each lysimeter was measured concurrently with N\textsubscript{2}O measurements using a portable pH meter (Mettler Toledo FiveGo\textsuperscript{TM} FG2) with a flat surface pH electrode (Mettler Toledo InLab\textsuperscript{®} Surface). Measurement of pH began on Thursday 21 July and continued to 19 January 2012 with a total of 17 measurements.

### 3.2.5.2 Sample analysis

Analyses of the headspace gas samples for N\textsubscript{2}O were performed using a gas chromatograph (model 8610; SRI Instruments, Torrance, CA) interfaced to a liquid autosampler (model 222XL; Gilson, Middleton, WI). The autosampler had been modified for gas analysis by substituting a purpose built (PDZ-Europa, Crewe, UK), double-concentric injection needle. This allowed the entire gas sample to be flushed rapidly from the vial into the gas chromatograph. The GC configuration included two 0.3 cm OD stainless steel columns packed with Haysep Q connected in series, oxygen free dry N carrier gas (40 mL min\textsuperscript{-1}) and a \textsuperscript{63}Ni electron capture detector at 320\textdegree C. Gas samples were generally analysed within one week of sampling. Immediately prior to analysis, the over-pressurised samples were brought to ambient atmospheric pressure by placing one end of a double ended hypodermic needle just below (0.5 cm) the surface of water in a small beaker, and piercing the vial’s septum with the other end. A brief flow of bubbles was observed in the water, and when this stopped, the gas in the vial was at ambient pressure. Dissipating this excess gas through a water medium gave a visual indication of when the samples were at ambient air pressure and also avoided any potential contamination of the sample with ambient air. Reference gases (0.32 \mu L L\textsuperscript{-1}, BOC Ltd, Auckland, New Zealand) were prepared using the same equilibration technique as described above. Detection limits for N\textsubscript{2}O analysis were considered significant if concentrations were 0.01 \mu L L\textsuperscript{-1} greater than the ambient concentration.

Analysis of the gas samples collected for \textsuperscript{15}N analysis was performed using an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20). The samples were analysed for N\textsubscript{2}O concentration and \textsuperscript{15}N enrichment. A detailed description of gaseous \textsuperscript{15}N analysis by IRMS is given in Section 3.2.8. The N\textsubscript{2}O-\textsuperscript{15}N recovered as a percentage of the total \textsuperscript{15}N applied as fertiliser was then calculated using Equation 3.2 above.

### 3.2.5.3 Calculation of gas flux from lysimeters

A total of three gas samples were collected from the chamber headspace of each lysimeter: one at t\textsubscript{0} (0 min), t\textsubscript{1} (30 min) and t\textsubscript{2} (60 min). This enabled the accurate
calculation of the $\text{N}_2\text{O}$ flux using Equations 3.3 and 3.4 described by Hutchinson and Mosier (1981) for both linear and non-linear $\text{N}_2\text{O}$ accumulation in the headspace.

The $\text{N}_2\text{O}$ flux ($F$) was calculated using the following equations:

$$F = \begin{cases} \frac{(C_1 - C_0)}{(C_2 - C_1)} \leq 1 & \rightarrow \text{Use linear equation 3.3} \\ \frac{(C_1 - C_0)}{(C_2 - C_1)} > 1 & \rightarrow \text{Use nonlinear equation 3.4} \end{cases}$$

$$F_{\text{Eq.3.3}} = \frac{(C_2 - C_0)}{(G_c (T_K + T_C) A_c t_2)}$$  \hspace{1cm} (3.3)

$$F_{\text{Eq.3.4}} = \frac{V_c (C_1 - C_0)^2}{(2C_1 - C_2 - C_0)} \ln \left[ \frac{C_1 - C_0}{C_2 - C_1} \right] \frac{P C_{ha} C_D M_{N_2}}{[G_c (T_K + T_C) A_c t_1]}$$  \hspace{1cm} (3.4)

Where:

$F$ = $\text{N}_2\text{O}$ flux [g $\text{N}_2\text{O}$-N ha$^{-1}$ day$^{-1}$]

$P$ = Atmospheric pressure [Pa] (101325)

$V_c$ = Chamber volume [m$^3$]

$A_c$ = Chamber area [m$^2$]

$G_c$ = Gas constant [J K$^{-1}$ mol$^{-1}$] (8.314)

$T_K$ = Absolute temperature at 0°C [K] (273.15)

$T_C$ = Air temperature at 5 cm height [°C]

$C_{ha}$ = Conversion factor m$^2$ to ha (10000)

$C_D$ = Minutes per day [min] (1440)

$M_{N_2}$ = Molecular weight of $\text{N}_2\text{O}$-N [g mol$^{-1}$] (28.0134)

$t_0$ = Time 0 minutes, start of cover period

$t_1$ = Time 30 minutes

$t_2$ = Time 60 minutes, total cover period

$C_0$, $C_1$, $C_2$ = $\text{N}_2\text{O}$ concentrations at time $t_0$, $t_1$, and $t_2$ [ppmv]

In order to calculate a cumulative $\text{N}_2\text{O}$ flux, a daily flux was calculated for the days where $\text{N}_2\text{O}$ was not measured by integrating the measured daily fluxes and linearly interpolating between each successive measurement (trapezoidal method). A cumulative $\text{N}_2\text{O}$ flux was then calculated by the addition of the measured and integrated fluxes.

3.2.6 Pasture collection and analysis

Lysimeter pasture cuts were carried out to imitate a typical grazing regime of a Waikato dairy system. In general, pasture was cut every 2 to 3 weeks during spring and autumn.
months, and every 3 to 4 weeks during summer and winter months. Pasture was cut to a height of 3 cm leaving a residual of approximately 1600 kg DM ha$^{-1}$ using an electric shearing clipper (Sunbeam Shearmaster 310C Head, USA) powered by a generator. The lysimeters that had not received any $^{15}$N urea were always cut first to prevent cross contamination of $^{15}$N. The herbage samples were oven-dried at 55°C and ground using a Cyclone Sample Mill with a 1 mm screen (UDY Corporation, USA). To minimise the potential for $^{15}$N contamination, control samples were ground first and the machine was cleaned between samples. The ground samples were analysed using an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20) for total N (%) and $^{15}$N atom %. The $^{15}$N recovered from pasture as a percentage of the total $^{15}$N applied as fertiliser was then calculated using Equation 3.2.

### 3.2.6.1 Pasture species composition

The lysimeter pasture was sprayed in February 2011 and 2012 with a selective herbicide (Preside™) to avoid the proliferation of weeds and ensure a predominantly ryegrass/clover sward. Pasture dissection was undertaken on the pasture harvested on 2 Jul 2012. The entire fresh pasture sample was mixed well and divided into 4 equal partitions on a well-lit dissection table. One quarter was kept for dissection and the remaining 3 quarters were returned to the paper bag for drying. The dissection was performed by separating generic clover, weed and grass species and placing them in separate labelled tins for oven drying at 40°C for 48 h. Once dried, all samples were weighed and the relative proportion of each species was calculated.

### 3.2.7 Soil collection and analysis

On 27 August 2012, all the lysimeters were destructively sampled. Nine cores, 2.5 cm diameter were taken to a depth of 70 cm (the total depth of the lysimeters). Six soil cores were designated for laboratory analysis and were cut and separated into depths of 0-5, 5-10, 10-15, 15-30, and 30-70 cm. The soil samples were stored, field moist, in plastic bags overnight in a chiller (< 4°C). The following morning, in preparation for laboratory analyses, these soils were passed through a 4 mm sieve, and any herbage material was removed.

The remaining three cores were designated for root and stubble analysis. The stubble was removed and stored separately, while the soil from the entire length of the three cores was
bulked together for root analysis. All soil samples were stored, field moist in plastic bags in a chiller (< 4°C) overnight.

**3.2.7.1 Gravimetric soil moisture**

Sub-samples of 5-10 g field moist sieved soil were weighed into small metal cups of a known weight, and oven-dried at 105°C for 24 hours. The samples were cooled in a dessicator, then re-weighed and the gravimetric water content was calculated as a percentage of the dry soil mass using Equation 3.5 (Topp and Ferré, 2002):

\[
\theta_g = 100 \times \left( \frac{M_w}{M_s} \right)
\]

Where \( \theta_g \) = gravimetric soil moisture content (g water per g oven dry soil); \( M_w \) = mass of water (g) (mass of field moist soil (g) – mass of oven dry soil (g)); and \( M_s \) = mass of oven dry soil (g).

**3.2.7.2 Soil mineral N**

The field moist equivalent of 10 g oven dry soil (approximately 15 g field moist soil) was extracted with 50 mL 0.5 \( M \) potassium sulphate (K\(_2\)SO\(_4\)) at 20°C on a reciprocating shaker (Mulvaney, 1996). The extractant was then filtered through fluted 12 cm Whatman #42 filter papers into 50 mL screw cap specimen vials and frozen until analysis. Blanks consisting of filtered K\(_2\)SO\(_4\) were also prepared. The extracts were analysed for ammonium (NH\(_4^+\)-N), and nitrate + nitrite (NO\(_3^-\)-N + NO\(_2^-\)-N) on the Skalar SAN++ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). Equation 3.6 was used to determine the inorganic N concentrations in the soil:

\[
N_S = \frac{N_e \times V}{M_s}
\]

Where \( N_s \) = inorganic N content (mg kg\(^{-1}\) dry soil); \( N_e \) = inorganic N concentration of soil extract (mg L\(^{-1}\)); \( V \) = volume of solution (K\(_2\)SO\(_4\) extract + soil moisture) (L); and \( M_s \) = mass of oven dry soil (kg).

**3.2.7.3 Soil microbial biomass N**

The chloroform fumigation extraction method (Brookes et al., 1985) was used to determine soil microbial biomass N. This method involved duplicate sub-samples of field moist soil being weighed out, one for fumigation, and the other non-fumigated sub-sample for immediate extraction with 0.5 \( M \) K\(_2\)SO\(_4\). However, the K\(_2\)SO\(_4\) extracts
prepared for soil mineral N analysis were also suitable “non-fumigated” extracts, therefore only the fumigated extracts required preparation.

Chloroform was first purified because commercial grade chloroform contains ethanol as a stabiliser, which is a C source (Jenkinson et al., 2004). The purification was carried out by shaking 100 mL of chloroform (Analar grade) with 200 mL of DI water in a 500 mL separating funnel. The water layer was discarded and the process was repeated twice more. The purified chloroform was stored in a stoppered glass bottle with anhydrous Na₂SO₄. Field moist soil (equivalent of 10 g oven dry soil) was weighed into aluminium cups. In a fume cupboard, the aluminium cups were positioned inside a dessicator along with a small conical flask containing 25 mL purified chloroform and ≈5 boiling chips. The lid was secured and the dessicator was evacuated using a vacuum pump until the chloroform was boiling – indicated by vigorous bubbling, at which point the dessicator was sealed and the vacuum pump removed. The dessicator was left inside a dark cupboard and the samples fumigated for at least 12 h. After this time, the dessicator was brought back to atmospheric pressure and the flask with any remaining liquid chloroform was removed. The dessicator was then re-evacuated and flushed three times with fresh air and left open inside the fume cupboard for 0.5 h to remove any remaining chloroform vapour. The soil samples were removed from the dessicator and re-weighed (as the fumigation process can slightly dry out the samples), after which they underwent extraction with 0.5 M K₂SO₄ as described in Section 3.2.7.2. The fumigated and non-fumigated extracts were analysed for total N by “oxidative combustion-chemiluminescence” using a Shimadzu TOC-VCSH/TNM-1 analyser and the fumigated samples were analysed for mineral N (NH₄⁺-N and NO₂⁻-N + NO₃⁻-N) on a Skalar SAN++ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). Organic N was calculated as the difference between total N and inorganic N. The microbial biomass N was calculated as the flush of organic N extracted from a fumigated soil less that extracted from a non-fumigated soil. The microbial biomass N was adjusted by a $k_{EN}$ factor of 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996), which accounts for the efficiency of extraction of organic microbial N after fumigation.

**3.2.7.4 Soil total N and ¹⁵N**

All soils were oven-dried at 50°C for 48 hours. A representative sub-sample was removed by riffling the dried soil. The sub-samples were then ground using a RockLabs orbital soil grinding machine CH-4 (Gilco Products, Albany, Auckland, New Zealand).
and analysed for total N and $^{15}$N enrichment on an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20). A detailed description of $^{15}$N analysis by IRMS is given in Section 3.2.8. Samples from lysimeters that did not receive $^{15}$N fertiliser treatments were riffled and ground first to minimise the risk of cross contamination of $^{15}$N. All equipment was also thoroughly cleaned between treatments.

The $^{15}$N recovered from the soil pool as a percentage of the total $^{15}$N applied, was then calculated using Equation 3.2.

### 3.2.7.5 Roots and stubble

The soil was rinsed off the root and stubble samples and they were oven dried at 55°C, weighed, recorded, and then finely ground using the Cyclone Sample Mill with 1 mm screen (UDY Corporation, USA). The ground samples were then analysed for total N and $^{15}$N enrichment using an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20). A detailed description of $^{15}$N analysis by IRMS is given in Section 3.2.8. The $^{15}$N recovered from the roots and stubble as a percentage of the total $^{15}$N applied, was calculated using Equation 3.2.

### 3.2.8 Isotope ratio mass spectrometry (IRMS) analysis

All $^{15}$N analyses were carried out using the EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry) at Lincoln University, New Zealand.

Solid materials that required $^{15}$N analysis (the dried filter discs from the diffusions, and the dried and ground plant and soil material), were weighed out and sealed in tin capsules, which were then placed into the auto-sampler of a PDZ Europa (Crewe, UK) GSL elemental analyser. Reference standards were also prepared in the same manner, at a rate of one reference for every eight samples, to check precision and accuracy. The reference material used was EM-WHEAT ($\delta^{15}$N$_{AIR} = 1.66 \%$), which had been normalised to the international reference material IAEA-N-1 (Ammonium Sulfate, $\delta^{15}$N$_{AIR} = 0.4 \%$). The samples were combusted in the presence of oxygen, converting the N in the sample to a mixture of NO$_x$ gases. These gases then pass through a packed copper column at 600°C, reducing the NO$_x$ species to N$_2$. The gas then flows through a CO$_2$ scrubber and a magnesium perchlorate Mg(ClO$_4$)$_2$ trap. The N$_2$ was then resolved on a gas chromatograph packed column. These columns have a large surface area and separate the gases depending on their molecular size and shape. Finally, the gas was passed into the
isotope ratio mass spectrometer (PDZ Europa 20-20 IRMS (Crewe, UK)), where masses 28, 29 and 30 were determined.

For the analysis of the gaseous N\sub{2}O-^{15}N samples, the 12 mL septum sealed sample vials were selected from the auto-sampler and flushed with helium gas through a double-ended needle. The majority of gas is flushed in a few seconds, however, the remainder bleeds out in about 12-15 seconds, resulting in a dilute spread of sample gas which does not form a distinguishable peak. To overcome this, the gas is passed into elliptical stainless steel cryotrap, which are lowered into liquid nitrogen (-196°C). The cryotrap freeze the initial sample gas in the column while allowing the remaining gas from the same sample to enter. The trap is then raised from the liquid nitrogen allowing the concentrated ‘slug’ of gas to carry on through CO\sub{2} and water traps prior to arriving on the gas chromatograph column, followed by the isotope ratio mass spectrometer (PDZ Europa 20-20 IRMS (Crewe, UK)) where masses 44, 45 and 46 were determined.

3.2.9 Mass balance

The fate of N was monitored via three key pathways throughout the duration of the experiment: leached N (inorganic and dissolved organic forms), N\sub{2}O emissions, and pasture N uptake. At the end of the experiment, the soil was destructively sampled and total N, inorganic N and microbial biomass N were measured.

Two mass balances were performed. A $^{15}$N recovery mass balance and an apparent mass balance of N applied as an apparent recovery, where the fate of N applied as urine and/or fertiliser was compared to the control with any differences attributed to the treatments applied.

Both of these mass balance approaches are presented because although the $^{15}$N recovery provides a reliable quantitative estimate of the fate of the enriched $^{15}$N applied, only the fertiliser was enriched in $^{15}$N, therefore it gives no indication of the fate of N in the treatments that did not receive fertiliser. Although the apparent N recovery is speculative, it gives an indication of the fate of N under both urine and fertiliser treatments.

3.2.9.1 Fertiliser $^{15}$N recovery balance

The recovery of $^{15}$N was calculated as the sum of the respective $^{15}$N recoveries from each fraction where it was measured (Equation 3.7).
Total $^{15}\text{N}$ recovery

$$= \sum \text{Leached} \, ^{15}\text{N} + \text{N}_2\text{O} - ^{15}\text{N} + \text{Uptake} \, ^{15}\text{N} + \text{Soil} \, ^{15}\text{N}$$  \hspace{1cm} \text{(3.7)}

Where: Total $^{15}\text{N}$ recovery is the total fertiliser $^{15}\text{N}$ recovered in each lysimeter as a percentage of the $^{15}\text{N}$ enriched fertiliser applied; Leached $^{15}\text{N}$ is the total fertiliser $^{15}\text{N}$ recovered in the leachate as a % of the enriched fertiliser applied; $\text{N}_2\text{O} \,-^{15}\text{N}$ is the total $^{15}\text{N}$ recovered as $\text{N}_2\text{O} \,-^{15}\text{N}$ as a % of the enriched fertiliser applied; Uptake $^{15}\text{N}$ is the total $^{15}\text{N}$ recovered in plant material (including pasture, roots and stubble) as a % of the enriched fertiliser applied; and Soil $^{15}\text{N}$ is the total $^{15}\text{N}$ recovered in the soil profile as a % of the enriched fertiliser applied.

An ANOVA was performed on the $^{15}\text{N}$ recovery mass balance data, and a least significant difference (LSD) was used to determine treatment effects.

3.2.9.2 Apparent N balance

The mass of N measured (or recovered) from each lysimeter was calculated using Equation 3.8:

$$\text{Apparent N recovery} = \sum \text{Leached} \, N + \text{N}_2\text{O} + \text{Pasture N}$$  \hspace{1cm} \text{(3.8)}

Where Apparent N recovery is the mass of the total apparent N recovery (kg N ha$^{-1}$); Leached $N$ is the cumulative total N leached (kg N ha$^{-1}$); $\text{N}_2\text{O}$ is the cumulative $\text{N}_2\text{O}$ emissions (kg $\text{N}_2\text{O}$-N ha$^{-1}$); and Pasture $N$ is the cumulative N uptake in pasture, over the entire experiment for each lysimeter. The apparent recovery of N in the soil pool was not included because the large background concentration and transient nature of N in this pool made apparent treatment effects difficult to identify. This mass of N recovered as a percentage of the treatment associated N applied can then be calculated using Equation 3.9:

$$\% \, \text{app N recovery} = \frac{\text{app N recovery(treatment)} - \text{app N recovery(control)}}{\text{N applied}} \times 100$$  \hspace{1cm} \text{(3.9)}

Where $\% \, \text{app N recovery}$ = the apparent N recovery as a percentage of the total N applied; app N recovery(treatment) = the mass N recovery from a given treatment (kg N ha$^{-1}$); app N recovery(control) = the mass N recovery from the control (kg N ha$^{-1}$); and N applied = the mass of N applied to the given treatment as urine and/or fertiliser (kg N ha$^{-1}$).
The calculation of the % apparent N recovery (Equation 3.9) subtracted the value of the control from the treatment values, and as such there is no apparent N recovery value for the control. To extract the effect of urine and urea, and their interaction, the data was analysed using the Residual Maximum Likelihood (REML) directive of GenStat (15th Edition) with the treatment effects determined by a Wald test.

3.2.10 Climate data

A range of daily climate data, including rainfall, temperature and soil moisture was collected over the entire course of the experiment from the Ruakura weather station (NIWA, 2007), located 0.1 km from the lysimeter site.

3.2.11 Statistical analysis

Unless specified differently, all statistical calculations were performed using the ANOVA directive of GenStat (15th Edition). The least significant difference (LSD) was used to determine statistical variation between the sample means at the 5% significance level. The LSD is presented in most graphical figures and tables. The “standard error of the difference between sample means” (SED) can be calculated by dividing the LSD (5%) by the t value (at the 5% significance level), or alternatively, a rough estimate of the SED can be calculated by dividing the LSD (5%) value by 2.

Where data was not normally distributed, it required transformation (to a log or rank scale) prior to statistical analysis. Where transformation was required, all conclusions are drawn from the analysis on the transformed scale; however, to maintain standard units, the means and error presented in tables and figures are from the backtransformed or untransformed data. Where data has been log transformed, the error is presented as the least significant ratio (LSR). On the log scale, a significant difference occurred when the ratio of the larger to a smaller mean was greater than the given LSR.
3.3 Results

Throughout the results and discussion sections, the nine treatments are referred to by their treatment names (Table 3.2). The different fertiliser rates, 0, 200 and 400 kg N ha\(^{-1}\) yr\(^{-1}\) are described hereafter as 0N, 200N and 400N, respectively.

3.3.1 Climate data

Rainfall was higher than the average annual Hamilton rainfall (1200 mm) during the experimental period. Total rainfall was 2,230 mm over the entire 19 month experiment, with 1410 mm rainfall in year one, and 820 mm in the remaining seven months. There was uncharacteristically high rainfall in December 2011 (224 mm) and early January 2012 (130 mm) (Figure 3.6). Mean monthly rainfall was 115 mm in 2011 and 100 mm in 2012. The highest monthly rainfall during the experiment occurred in December 2011 (224 mm) and the lowest in August 2011 (27.6 mm). The maximum daily rainfall occurred on 26 May 2011 with 71 mm.

The average mean daily air temperature over the entire experiment was 12.6°C ranging from 1.0-23.5°C (Figure 3.6) and the minimum and maximum temperatures were -4.5 and 29.8°C, respectively. The average mean daily soil temperature at 10 cm depth was 13.9°C, ranging from 4.5-22°C. There were 39 frost days (16 in 2011, and 23 in 2012).

![Figure 3.6 Daily rainfall, mean air and soil (10 cm depth) temperatures and evapotranspiration at Ruakura over the duration of the experiment.](image-url)
3.3.2 Leachate

3.3.2.1 Drainage

A total of 21 leachate collections (12 during 2011 and 9 during 2012) were made throughout the experiment. Mean cumulative drainage ranged from 843-1148 mm (Figure 3.7a). Mean cumulative drainage as a % of cumulative rainfall ranged from 43-58% (Figure 3.7b). In 2011, drainage commenced in late March, and continued until October 2011. Cumulative drainage over this time was between 420 and 520 mm, however, the majority of the drainage (310-370 mm) occurred between 30 Apr and 15 Jul 2011. A series of high rainfall events in late December 2011 and early January 2012 resulted in uncharacteristic summer drainage of 90-160 mm, requiring two leachate collections over this time. In 2012, drainage commenced in mid-April and finished in mid-September.

There was a fertiliser treatment effect (p < 0.001) on the total cumulative drainage where treatments with the 0N fertiliser rate had the greatest cumulative drainage, followed by the 200N rate, then the 400N rate. There was also a urine treatment effect (p < 0.001) where the nil urine treatments had the greatest cumulative drainage, followed by the autumn urine, then spring urine treatments. These treatment effects became evident from August 2011 (Figure 3.7). There was no interaction between fertiliser and urine on drainage.
3.3.2.2 Leached inorganic NH$_4^+$-N

The leached inorganic NH$_4^+$-N data was rank transformed prior to statistical analysis, to account for large variability. All conclusions are drawn from the analysis on the transformed scale. The means and error presented in figures are of the untransformed data.

Ammonium concentrations in the leachate were < 3 mg NH$_4^+$-N L$^{-1}$ in all treatments throughout the experiment with three exceptions on 28 Mar, 23 May and 4 Oct 2011 (Figure 3.8b and Figure 3.9a). These included mean concentrations of 5, 9 and 10 mg NH$_4^+$-N L$^{-1}$ from the F4US, F2UA and F4UA treatments, on 28 Mar, 23 May and 4 Oct...
2011 (15, 100 and 485 mm drainage), respectively. The peaks of 9 and 10 mg NH$_4^+$-N L$^{-1}$ in treatments F2UA and F4UA occurred 18 and 153 days after autumn urine application. There was no urine or fertiliser effect on leached NH$_4^+$-N concentrations, nor any urine-fertiliser interaction.

Cumulative NH$_4^+$-N leached was < 1 kg NH$_4^+$-N ha$^{-1}$ in all but three treatments (Figure 3.9a and Figure 3.9b). Treatments F4UA, F0US and F2UA cumulatively lost 2.0, 2.1 and 4.5 kg NH$_4^+$-N ha$^{-1}$, respectively over the course of the experiment. However, the variation between treatment means was large, and the cumulative NH$_4^+$-N leached did not differ significantly with any treatment. There was no urine or fertiliser effect on leached NH$_4^+$-N concentrations, nor any urine-fertiliser interaction.

Figure 3.8  Mean NH$_4^+$-N concentration vs (a) cumulative drainage and (b) time, from 28 Mar 2011 to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), n = 4.
3.3.2.3 Leached inorganic NO$_3^-$-N

To account for large variability, the leached inorganic NO$_3^-$-N data was rank transformed prior to statistical analysis. All conclusions are drawn from the analysis on the transformed scale. The means and error presented in figures are of the untransformed data.

In the nil urine treatments, NO$_3^-$-N concentrations were < 2.5 mg L$^{-1}$ from the commencement of the experiment to 19 Dec 2011, or 565 mm drainage (Figure 3.10a and Figure 3.10b). On 11 Jan 2012 (640 mm drainage) the NO$_3^-$-N concentration in treatment
F4U0 peaked at 11.5 mg NO$_3^{-}$N L$^{-1}$ while the F0U0 and F2U0 treatments remained at $< 1$ mg NO$_3^{-}$N L$^{-1}$. Treatment F4U0 returned to background levels by 19 Jan 2012 (642 mm drainage).

In all the autumn urine treatments, NO$_3^{-}$N concentrations peaked at 205 mg NO$_3^{-}$N L$^{-1}$ on 8 Aug 2011, regardless of fertiliser rate, 96 days after autumn urine application (Figure 3.10b) or 460 mm drainage (Figure 3.10a). This peak occurred after 360 mm drainage had occurred since urine application, and before one pore volume of drainage (490 mm) occurred. Concentrations returned to background levels by 11 Jan 2012 (640 mm drainage), 252 days after autumn urine application and remained there for the remainder of the experiment.

Leachate NO$_3^{-}$N concentrations in the spring urine treatments did not increase from background levels until after the 2011 winter drainage season and peaked at different times. Peaks of 30, 23 and 41 mg NO$_3^{-}$N L$^{-1}$ occurred in treatments F0US, F2US and F4US, respectively, on 11 Jan 2012, 30 May 2012, and 11 Jul 2012 (Figure 3.10b) or 640, 672 and 760 mm drainage (Figure 3.10a), respectively. These peaks occurred 133, 273 and 315 days after spring urine application for F0US, F2US and F4US, respectively, and returned to background levels by 19 Apr 2012, 27 Aug 2012 and 27 Aug 2012 (642, 1010 and 1010 mm drainage), 232, 362 and 362 days after urine application, respectively.

From the time of spring urine application, these peaks occurred before one pore volume of drainage had occurred.

There was a urine treatment effect ($p < 0.001$) on all days from 30 May 2011 (140 mm drainage) to 11 Jan 2012 (524 mm drainage) where NO$_3^{-}$N concentrations in leachate from the autumn urine treatments was greater than those in the spring urine and nil urine treatments. This urine effect continued ($p < 0.05$) up to 3 Aug 2012 (750 mm drainage). The peak NO$_3^{-}$N concentrations leached under all autumn urine treatments were greater ($p < 0.001$) than those under the spring urine treatments, which were themselves greater than those under the nil urine treatments.

There was a fertiliser treatment effect ($p < 0.01$) on NO$_3^{-}$N leached from 24 Jun 2011 to 8 Aug 2011 (300 to 460 mm drainage), where more NO$_3^{-}$N was leached from the 400N and 0N treatments than the 200N treatments. In addition, from 30 May 2012 to 16 Aug 2012 (670 to 990 mm drainage), NO$_3^{-}$N leached was greater ($p < 0.01$) from the 400N and 200N treatments than the 0N treatments (Figure 3.10b).
There was no consistent interaction between the urine and fertiliser treatments on leached NO$_3^-$-N concentrations.

![Figure 3.10 Mean NO$_3^-$-N concentrations in leachate vs (a) drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.](image)

Cumulative NO$_3^-$-N leached was greatest in the autumn urine treatments ($p < 0.001$), with mean totals of 325, 280 and 380 kg NO$_3^-$-N ha$^{-1}$ for the F0UA, F2UA and F4UA treatments, respectively (Figure 3.11a and Figure 3.11b). In the spring urine treatments, mean cumulative NO$_3^-$-N values were 28, 30 and 75 kg NO$_3^-$-N ha$^{-1}$ from the F0US, F2US and F4US treatments, respectively and were greater ($p < 0.001$) than the cumulative NO$_3^-$-N leached under the nil urine treatment, which was $< 8$ kg NO$_3^-$-N ha$^{-1}$. 
There was a significant fertiliser effect at the 400N rate under autumn and spring urine, where the cumulative NO$_3$-N leached was greater ($p < 0.01$) than under urine alone. There was no fertiliser effect at the 200N fertiliser rate on cumulative NO$_3$-N leached, nor was there any interaction between urine and fertiliser.

Figure 3.11 Cumulative NO$_3$-N leached with (a) cumulative drainage and (b) time, from 28 Mar 2011 to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), $n = 4$.

3.3.2.4 Leachate inorganic $^{15}$N recovery

To account for skewness, the recovery of the $^{15}$N labelled fertiliser in the inorganic N leachate pool was log transformed prior to statistical analysis. All conclusions are drawn
from the analysis of data on the transformed scale. The means and error presented in figures are of the non-transformed data.

The recovery of the $^{15}$N labelled fertiliser in the inorganic N leachate pool was $< 5\%$ across all treatments. The vast majority of this $^{15}$N was recovered as NO$_3^-$-N (Figure 3.12). The $^{15}$N recovered as NH$_4^+$-N was negligible, and is not presented here. Each point on Figure 3.12 represents the cumulative $^{15}$N recovered between each ensuing $^{15}$N enriched fertiliser application (with the exception of the first point, which includes the first 2 fertiliser applications as there was insufficient NO$_3^-$-N concentrations in the leachate collected up until this time).

In the autumn urine treatments, mean cumulative NO$_3^-$-$^{15}$N recovery increased between the 3$^{rd}$ and 4$^{th}$ fertiliser application, reaching 2.5 and 4.5$\%$ in F2UA and F4UA, respectively. Thereafter, the mean $^{15}$N recovery in these treatments trended downwards due to the ensuing fertiliser applications, resulting in a total of 1.4 and 1.8$\%$ $^{15}$N recovery, respectively, by 1 Jun 2012. In the case of the spring urine treatments, mean cumulative recovery remained below 0.2$\%$ until 30 May 2012, after which, they increased to 0.7 and 2.2$\%$ for F2US and F4US, respectively. Mean cumulative recovery of fertiliser $^{15}$N as NO$_3^-$-$^{15}$N from the nil urine treatments remained negligible at $< 0.15\%$ (Figure 3.12).

There was a significant urine effect on every day except 4 May 2011, where the recovery of fertiliser $^{15}$N as NO$_3^-$-$^{15}$N was greater ($p < 0.001$) in the autumn urine treatments than the spring urine and nil urine treatments. On 31 May 2012, fertiliser $^{15}$N recovery increased from the F4US treatment and this too, was greater ($p < 0.001$) than the F2US treatment and the nil urine treatments (Figure 3.12).

There was no significant fertiliser rate effect nor was there a urine and fertiliser interaction on the recovery of the $^{15}$N labelled fertiliser in the inorganic N leachate pool.
Figure 3.12  Mean cumulative fertiliser $^{15}$N recovery as NO$_3$--$^{15}$N in the leachate, from 21 Feb 2011 to 28 Aug 2012 for treatments that received $^{15}$N labelled fertiliser. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine, respectively. Bold arrows indicate urine + fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.

3.3.2.5 Leached dissolved organic N (DON)

To account for large variability, the leached DON data was rank transformed prior to statistical analysis. All conclusions are drawn from the analysis on the transformed scale. The means and error presented in figures are of the untransformed data.

In the nil urine treatments, mean DON concentrations in the leachate remained $< 2$ mg L$^{-1}$. In the autumn urine treatments, mean DON concentrations peaked on 8 Aug 2011 (Figure 3.13b) (170 mm drainage, Figure 3.13a) at 61, 56 and 63 mg N L$^{-1}$ in treatments F0UA, F2UA and F4UA, respectively. These peaks occurred 96 days following autumn urine application and returned to control levels by 19 Dec 2011 (465 mm drainage), 230 days following autumn urine application. In treatment F2UA only, prior to the main peak, a smaller peak of 13 mg N L$^{-1}$ occurred on 23 May 2011 (Figure 3.13b) (92 mm drainage, Figure 3.13a), 19 days following autumn urine application. This peak returned to control levels by the following leachate collection on 30 May 2011. From 19 Dec 2011 onwards, DON concentrations in all autumn urine treatments remained at control levels.
In the spring urine treatments there was no clear peak across all treatments. Mean DON concentrations remained at background levels until 5 Oct 2011 (226 mm drainage) when there was a small increase in concentrations to 4.9, 0.5 and 2.2 mg N L$^{-1}$ in the F0US, F2US and F4US treatments, respectively (Figure 3.13a and Figure 3.13b) returning to control levels by 27 Oct 2011 (250 mm drainage). On 31 May 2012 (465 mm drainage), the DON concentration in treatment F2US increased to 3.7 mg N L$^{-1}$, nine months following the spring urine application; and on 23 Jul 2012 (520 mm drainage), the DON concentration in treatment F4US increased to 8.6 mg N L$^{-1}$, 11 months following spring urine application. These peaks both returned to control levels by 3 Aug 2012 (530 mm drainage).

There was a significant urine effect on leached DON concentrations from 13 Jun 2011 to 27 Jul 2012, where from 13 Jun to 19 Dec 2011 the DON concentrations under autumn urine were greater ($p < 0.001$) than under spring urine and nil urine treatments; and from 19 Dec to 27 Jul 2012 the DON concentrations under spring urine were greater ($p < 0.05$) than under autumn urine and nil urine. There were no consistent fertiliser effects on leached DON due to high variation of the data, however, there was a trend that under the autumn urine treatments, DON concentrations were generally greater under the 400N and 0N rates than the 200N rate, and under the spring urine treatments DON concentrations were greater under the 400N rate, followed by the 200N rate, then the 0N rate (Figure 3.13a and Figure 3.13b).

There was a urine and fertiliser interaction on 23 Jul 2012 where under spring urine, DON concentrations were greater ($p < 0.05$) under the 400N fertiliser rate, followed by the 200N rate, followed by the 0N rate; however, there was no difference between fertiliser rates on DON concentrations from the autumn urine treatments. No other urine and fertiliser interactions occurred.
Figure 3.13  Mean leached DON concentrations vs (a) cumulative drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n = 4.

Cumulative DON leached from autumn urine treatments equated to 82, 62 and 87 kg DON ha\(^{-1}\) from the F0UA, F2UA and F4UA treatments, respectively. These were greater (p < 0.001) than those from the spring urine and nil urine treatments (Figure 3.14a and Figure 3.14b). There was no difference in cumulative DON leached between the F4UA and F0UA treatments, but these were both greater than the cumulative DON leached from the F2UA (p < 0.05) treatment. The majority of DON leached from the autumn urine treatments was lost between 23 May 2011 and 8 Aug 2011 (Figure 3.14b), (92 and 170 mm drainage, Figure 3.14a).
Cumulative DON leached from the spring urine treatments was 3.3, 2.5 and 12 kg DON ha\(^{-1}\) from the F0US, F2US and F4US treatments, respectively; and cumulative DON leached from all nil urine affected treatments was < 1.1 kg DON ha\(^{-1}\). There was no statistical difference between the cumulative DON leached from the spring urine and nil urine treatments, and no fertiliser effect on DON leached for any urine treatment. There was also no interaction between the urine and fertiliser treatments on the cumulative DON leached.

**Figure 3.14** Mean cumulative leached DON concentrations vs (a) cumulative drainage and (b) time, from 28 Mar to 28 Aug 2012 for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), \(n = 4\).
3.3.3 Nitrous oxide

3.3.3.1 Temporal N₂O

Nitrous oxide flux data did not follow a normal distribution and required log transformation prior to statistical analysis. Raw N₂O-N flux data that was less than or equal to zero was adjusted to a value of 0.001 g N₂O-N ha⁻¹ d⁻¹ (half the minimum recorded flux) prior to log transformation. To maintain standard units, the back-transformed means of the N₂O flux data are presented (in Figure 3.15 and Figure 3.16). The error is presented as the least significant ratio (LSR). On the log scale, a significant difference occurred when the ratio of the larger to a smaller mean was greater than the given LSR.

Nitrous oxide fluxes generally increased within 2 days following urine and/or fertiliser applications (Figure 3.15). In the nil urine treatments, mean N₂O-N fluxes ranged from <0.2-20, 3-39, and 4-41 g N₂O-N ha⁻¹ d⁻¹ in the F0U0, F2U0 and F4U0 treatments, respectively. These peak N₂O-N fluxes returned to control levels within ~ 1 week (Figure 3.15a). On 26 May 2011 there were non-fertiliser associated peaks of 20 and 39 g N₂O-N ha⁻¹ d⁻¹ in the F0U0 and F2U0 treatments, respectively, and only 6 g N₂O-N ha⁻¹ d⁻¹ in the F4U0 treatment. With the exception of the single flux of 20 g N₂O-N ha⁻¹ d⁻¹ on 26 May 2011, there were no other instances of N₂O fluxes in the F0U0 treatment increasing above 6 g N₂O-N ha⁻¹ d⁻¹.

There was a significant fertiliser effect on N₂O emissions 2 days after the first fertiliser application (21 Feb 2011); 6, and 8 days after the third fertiliser application with autumn urine (4 May 2011); 1, 4, 8 and 24 days after the fourth fertiliser application (31 May 2011); 14 days after the fifth fertiliser application with spring urine (31 August 2011); 4 days after the sixth fertiliser application; 3 days after the seventh fertiliser application (7 Nov 2011); and 3 days after the eighth fertiliser application (5 Dec 2011); where N₂O-N fluxes were greater (p < 0.05) from the treatments, within each urine timing, that received the 400N fertiliser rate, compared to the 200N fertiliser rate. The mean N₂O-N fluxes in the fertiliser treatments are shown in Table 3.4 along with the error and the level of significance (P value) of the fertiliser rate effect.
Table 3.4 Occurrences of significant fertiliser treatment effects on N$_2$O-N fluxes relative to the fertiliser application date.

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after last fert application$^1$</th>
<th>F2U0</th>
<th>F2UA</th>
<th>F2US</th>
<th>F4U0</th>
<th>F4UA</th>
<th>F4US</th>
<th>LSR (5%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-02-11</td>
<td>2 (#1)</td>
<td>5.92</td>
<td>4.42</td>
<td>5.29</td>
<td>8.58</td>
<td>15.2</td>
<td>9.84</td>
<td>1.99</td>
<td>0.001</td>
</tr>
<tr>
<td>10-05-11</td>
<td>6 (#3)</td>
<td>3.50</td>
<td>53.1</td>
<td>4.31</td>
<td>7.81</td>
<td>53.3</td>
<td>4.92</td>
<td>1.53</td>
<td>0.016</td>
</tr>
<tr>
<td>12-05-11</td>
<td>8 (#3)</td>
<td>4.41</td>
<td>210</td>
<td>4.60</td>
<td>11.2</td>
<td>143</td>
<td>8.43</td>
<td>1.9</td>
<td>0.044</td>
</tr>
<tr>
<td>01-06-11</td>
<td>1 (#4)</td>
<td>2.32</td>
<td>23.5</td>
<td>3.12</td>
<td>4.42</td>
<td>32.3</td>
<td>3.24</td>
<td>1.68</td>
<td>0.032</td>
</tr>
<tr>
<td>03-06-11</td>
<td>4 (#4)</td>
<td>1.55</td>
<td>18.0</td>
<td>1.65</td>
<td>3.21</td>
<td>19.1</td>
<td>2.80</td>
<td>1.95</td>
<td>0.028</td>
</tr>
<tr>
<td>07-06-11</td>
<td>8 (#4)</td>
<td>5.99</td>
<td>28.4</td>
<td>6.32</td>
<td>12.0</td>
<td>41.7</td>
<td>8.42</td>
<td>1.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>23-06-11</td>
<td>24 (#4)</td>
<td>1.22</td>
<td>6.90</td>
<td>0.72</td>
<td>2.58</td>
<td>10.4</td>
<td>1.18</td>
<td>1.96</td>
<td>0.009</td>
</tr>
<tr>
<td>13-09-11</td>
<td>14 (#5)</td>
<td>2.54</td>
<td>7.4</td>
<td>88.1</td>
<td>14.5</td>
<td>39.8</td>
<td>53.8</td>
<td>5.08</td>
<td>0.043</td>
</tr>
<tr>
<td>03-10-11</td>
<td>4 (#6)</td>
<td>6.89</td>
<td>8.74</td>
<td>105</td>
<td>23.2</td>
<td>42.4</td>
<td>84.0</td>
<td>3.69</td>
<td>0.029</td>
</tr>
<tr>
<td>10-11-11</td>
<td>3 (#7)</td>
<td>13.7</td>
<td>25.6</td>
<td>30.6</td>
<td>22.6</td>
<td>28.4</td>
<td>50.9</td>
<td>1.67</td>
<td>0.017</td>
</tr>
<tr>
<td>08-12-11</td>
<td>3 (#8)</td>
<td>2.40</td>
<td>12.8</td>
<td>4.50</td>
<td>3.41</td>
<td>11.4</td>
<td>10.9</td>
<td>1.78</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^1$ Values in parentheses indicate the number of the split fertiliser application

Following the application of autumn urine on 4 May 2011, mean N$_2$O-N fluxes peaked after seven days at 310, 210, and 143 g N$_2$O-N ha$^{-1}$ d$^{-1}$ in treatments F0UA, F2UA and F4UA, respectively, that is, the greatest peak occurred under the 0N fertiliser rate, followed by the 200N rate, followed by the 400N rate. These peaks returned to control levels 26 days after urine application (Figure 3.15b). After the N$_2$O peak associated with autumn urine application, there were smaller, fertiliser associated peaks that followed a similar pattern as previously described for the nil urine treatments.

Prior to spring urine application, there were fertiliser associated peaks that followed a similar pattern as described in the nil urine treatments above. There was a large flux (50 g N$_2$O-N ha$^{-1}$ d$^{-1}$) from treatment F0US on 14 Apr 2011 (returning to control levels within 1 week), yet no elevated N$_2$O-N fluxes in the other spring urine treatments. On 26 May 2011, similarly to the nil urine treatments, mean N$_2$O-N fluxes peaked at 34, 16 and 88 g N$_2$O-N ha$^{-1}$ d$^{-1}$ from treatments F0US, F2US and F4US, respectively. Following spring urine application, there were 2 major N$_2$O-N peaks. The first occurred 13 days after application with mean fluxes of 81, 88 and 55 g N$_2$O-N ha$^{-1}$ d$^{-1}$ for the F0US, F2US and
F4US treatments, respectively. The second occurred 33 days after urine application with mean fluxes of 141, 104 and 83 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1} for the F0US, F2US and F4US treatments, respectively. Again, the greatest peak occurred under the 0N fertiliser rate, followed by the 200N rate, followed by the 400N rate. All urine associated peaks returned to control levels within 40 days of spring urine application (Figure 3.15c). After these peaks, the fertiliser associated peaks resumed in the remaining measurements, in the same fashion as described in the nil urine treatments earlier.

There was a significant urine effect from 5 May 2011 where the N\textsubscript{2}O-N emissions from the autumn urine treatments were greater (p < 0.001) than the spring urine (where no urine had as yet been applied) and nil urine treatments. Following spring urine application, from 1 Sep 2001 to 3 Oct 2012, the N\textsubscript{2}O-N fluxes from the spring urine treatments were greater (p < 0.001) than those from the autumn urine and nil urine treatments. From 3 Oct 2011 onwards there remained a significant urine effect where N\textsubscript{2}O-N fluxes from the urine treatments (spring and autumn) were greater (p < 0.05) than from the nil urine treatments.

Some N\textsubscript{2}O-N flux peaks occurred during or shortly after rainfall events (usually > 10 mm day\textsuperscript{-1}) and subsequent increases in soil moisture (Figure 3.16). The key rainfall events, i.e. those that resulted in increased N\textsubscript{2}O-N fluxes across most treatments, included: 26 Mar 2011 (39 mm), 5 Apr 2011 (13 mm), 26 Apr 2011 (50 mm), 26 May 2011 (71 mm), 10 Jun 2011 (38 mm), 11 Sep 2011 (17 mm), 3 Oct 2011 (24 mm), 4 Dec 2011 (13 mm), 15 Dec 2011 (22 mm) and 15 Feb 2012 (4 mm) (Figure 3.16). Rainfall of up to 20 mm occurred every day from 6 to 14 Jul 2011 resulting in a large increase in soil moisture. This resulted in increased N\textsubscript{2}O-N fluxes in the autumn urine treatments, post rainfall (Figure 3.16b), but not in the nil urine or spring urine treatments (Figure 3.16a and Figure 3.16b). However, a series of large rainfall events between 17 Dec 2011 and 8 Jan 2012 did not result in any notable increases in N\textsubscript{2}O-N fluxes from any treatments.

Some minor N\textsubscript{2}O flux peaks also occurred throughout the study period that were not associated with fertiliser or urine application, or rainfall events. On 28 Oct 2011 mean fluxes ranged from 3 to 9 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1} in the nil urine treatments, and were all approximately 21 and 24 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1} in the autumn and spring urine treatments, respectively. Also on 15 Feb 2012, mean N\textsubscript{2}O-N fluxes of 8, 18 and 21 occurred under nil, autumn and spring urine, respectively yet only 4 mm rainfall occurred prior to this.
Figure 3.15 Mean temporal N₂O emissions, from 21 Feb 2011 to 28 Feb 2012 from (a) nil urine, (b) autumn urine and (c) spring urine treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), error is only shown if LSR is > 5, n = 4. Note different y axis scales.
Figure 3.16  Mean temporal N$_2$O emissions, daily rainfall and soil moisture, from 21 Feb 2011 to 28 Feb 2012 from (a) nil urine (b) autumn urine and (c) spring urine treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSR (5%), error is only shown if LSR is > 5, n = 4. Note different y axis scales.
3.3.3.2 Cumulative N₂O

Mean cumulative N₂O fluxes were highest in the autumn urine treatments (p < 0.001), and were 5300, 6100 and 6400 g N₂O-N ha⁻¹ for the F0UA, F2UA and F4UA treatments, respectively (Figure 3.17). In the month following autumn urine application, mean cumulative N₂O emissions from the affected lysimeters increased from 460 to 2700 g N₂O-N ha⁻¹ (Figure 3.17). The spring urine treatments were next highest (p < 0.001) with mean cumulative fluxes of 3800, 4100 and 4300 g N₂O-N ha⁻¹ from the F0US, F2US and F4US treatments, respectively. In the month following spring urine application, mean cumulative N₂O emissions from the affected lysimeters also increased, from 870 to 2700 g N₂O-N ha⁻¹. The nil urine treatments had the lowest mean cumulative N₂O emissions at 800, 1500 and 1700 g N₂O-N ha⁻¹ from the F0U0, F2U0 and F4U0 treatments, respectively. There was no fertiliser effect and no interaction of fertiliser and urine on cumulative N₂O emissions (Figure 3.17).

Figure 3.17 Mean cumulative N₂O emissions, from 21 Feb 2011 to 28 Feb 2012 from all treatments. F0, F2 and F4 = fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US = nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error = LSD (5%), n = 4.

The cumulative N₂O fluxes are also expressed as a percentage of the total N applied, and as an emission factor (Table 3.5) which was calculated using Equation 3.10.

\[
EF = \frac{Cum \text{ N}_2\text{O}-\text{N}(\text{treatment}) - Cum \text{ N}_2\text{O}-\text{N}(\text{control})}{N \text{ applied}_{mass}} \times 100 \tag{3.10}
\]
Where $EF$ = the emission factor ($\%$); $Cum N_2O-N(\text{treatment})$ = the cumulative $N_2O$ emissions for a given treatment (g $N_2O-N$ ha$^{-1}$); $CumN_2O-N(\text{control})$ = the cumulative $N_2O$ emissions from the F0U0 treatment (g $N_2O-N$ ha$^{-1}$) and $N \text{ applied mass}$ = the amount, in kg N ha$^{-1}$ of N applied as urine (800 kg N ha$^{-1}$) and/or fertiliser (according to treatment).

Table 3.5 Cumulative $N_2O-N$ fluxes for each treatment expressed as a % of total N applied, and as an emission factor.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Cumulative $N_2O-N$ flux (g $N_2O-N$ ha$^{-1}$)</th>
<th>Cumulative $N_2O-N$ flux (% N applied)</th>
<th>Emission Factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0U0</td>
<td>0N urea, nil urine</td>
<td>766.1 (a)</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>F2U0</td>
<td>200N urea, nil urine</td>
<td>1470 (a)</td>
<td>0.74 (a)</td>
<td>0.352 (a)</td>
</tr>
<tr>
<td>F4U0</td>
<td>400N urea, nil urine</td>
<td>1732 (a)</td>
<td>0.43 (b)</td>
<td>0.241 (b)</td>
</tr>
<tr>
<td>F0UA</td>
<td>0N urea, autumn urine</td>
<td>5292 (bc)</td>
<td>0.66 (ac)</td>
<td>0.566 (ac)</td>
</tr>
<tr>
<td>F2UA</td>
<td>200N urea, autumn urine</td>
<td>6104 (b)</td>
<td>0.61 (ab)</td>
<td>0.534 (ac)</td>
</tr>
<tr>
<td>F4UA</td>
<td>400N urea, autumn urine</td>
<td>6436 (b)</td>
<td>0.54 (ab)</td>
<td>0.473 (bc)</td>
</tr>
<tr>
<td>F0US</td>
<td>0N urea, spring urine</td>
<td>4144 (c)</td>
<td>0.52 (bc)</td>
<td>0.422 (bc)</td>
</tr>
<tr>
<td>F2US</td>
<td>200N urea, spring urine</td>
<td>3785 (c)</td>
<td>0.38 (b)</td>
<td>0.302 (b)</td>
</tr>
<tr>
<td>F4US</td>
<td>400N urea, spring urine</td>
<td>4332 (c)</td>
<td>0.36 (b)</td>
<td>0.297 (b)</td>
</tr>
<tr>
<td>LSD (5%)$^1$</td>
<td></td>
<td>1657</td>
<td>0.20</td>
<td>0.194</td>
</tr>
</tbody>
</table>

$^1$Different letters indicate a significant difference between treatments at the 5% ($p < 0.05$) level.

### 3.3.3.3 Temporal $N_2O-^{15}N$ recovery

Recovery of fertiliser $^{15}N$ as $N_2O$ increased within 2 days of each fertiliser and/or urine application (Figure 3.18). Recovery was greatest (0.00084%) following the first fertiliser application (21 Feb 2011) and returned to baseline levels 15 days later. The remaining fertiliser and/or urine associated peaks ranged from 0.00015-0.00054% and returned to baseline levels within one to two weeks, the exception being after autumn urine, which returned to baseline after six weeks.

Recovery of $^{15}N$ as $N_2O$ was generally greater from the treatments that received the 400N fertiliser rate compared to the 200N rate. However, this recovery was statistically greater on only three occasions: after the first fertiliser application ($p < 0.05$), after the autumn urine + fertiliser application ($p < 0.05$), and during winter drainage from 8 Jul 2011 to 4 Aug 2011 ($p < 0.05$).
There was no significant interaction between urine and fertiliser treatments on fertiliser $^{15}\text{N}$ recovery as $\text{N}_2\text{O-}^{15}\text{N}$ (p > 0.05) on a temporal basis. The increase in $\text{N}_2\text{O-}^{15}\text{N}$ recovery was larger after the autumn urine application (up to 0.0054%) compared to after the spring urine application (up to 0.0016%). However, fertiliser $^{15}\text{N}$ recovery as $\text{N}_2\text{O-}^{15}\text{N}$ increased with successive fertiliser applications following the spring urine application (Figure 3.18).

Figure 3.18  Mean recovery of $\text{N}_2\text{O-}^{15}\text{N}$ from treatments that received $^{15}\text{N}$ labelled fertiliser, from 21 Feb 2011 to 28 Feb 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + $^{15}\text{N}$ fertiliser application, other arrows indicate split fertiliser $^{15}\text{N}$ applications. Error bars = LSD (5%), n = 4.

3.3.3.4 Cumulative $\text{N}_2\text{O-}^{15}\text{N}$ recovery

The highest mean cumulative fertiliser $^{15}\text{N}$ recoveries as $\text{N}_2\text{O-}^{15}\text{N}$ were 0.040, 0.039 and 0.036 % from the F4UA, F4U0 and F2UA treatments, respectively. All other treatments had a mean cumulative recovery of approximately 0.025% (Figure 3.19). There were two occasions where clear increases in cumulative fertiliser $^{15}\text{N}$ recovery as $\text{N}_2\text{O-}^{15}\text{N}$ occurred during the experiment; the first was after autumn urine was applied (4 May 2011) to 16 June 2011, where cumulative $\text{N}_2\text{O-}^{15}\text{N}$ recovery increased by 0.014, 0.011 and 0.009% from the F4UA, F4U0 and F2UA treatments; and increases of 0.006, 0.006 and 0.003% from the F4US, F2US and F2U0 treatments, respectively. The second event was from 15
Nov 2011 to 11 Jan 2012 where treatments F4UA, F4U0 and F2UA increased by 0.01% and the remaining treatments increased by 0.007% (Figure 3.19).

Cumulative N$_2$O-$^{15}$N recovery varied with urine treatment (p < 0.05) where autumn applied urine resulted in the greater N$_2$O-$^{15}$N recovery than spring applied urine, while nil urine application resulted in an intermediary cumulative N$_2$O-$^{15}$N recovery (Figure 3.19). There was no interaction between the fertiliser and urine treatments on fertiliser $^{15}$N recovery as cumulative N$_2$O-$^{15}$N. While fertiliser rate did not statistically affect cumulative N$_2$O-$^{15}$N recovery, there was a trend for N$_2$O-$^{15}$N recovery to increase with increasing fertiliser rate (p = 0.052) (Figure 3.19).

![Figure 3.19 Mean cumulative recovery of N$_2$O-$^{15}$N from $^{15}$N affected treatments from 21 Feb 2011 to 28 Feb 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + $^{15}$N fertiliser application, other arrows indicate split $^{15}$N fertiliser applications. Error bar = LSD (5%), n = 4.](image)

3.3.3.5 Surface soil pH

Soil surface pH was only measured from 21 July 2011 to 19 Jan 2012. Control soil surface pH values ranged between 5.5 and 6.0 (Figure 3.20). Soil pH rapidly increased (p < 0.001) following the spring urine application on 31 Aug 2011 from control levels to 8.0, 8.0 and 8.0 in the F0US, F2US and F4US treatments, respectively (Figure 3.20). A significant spring urine effect remained until 8 Sep 2011, where pH was greater (p <
0.001) under the spring urine treatments, then on 28 Sep 2011, pH was lower (p < 0.05) in the spring urine treatments.

There was a significant fertiliser effect on soil surface pH (p < 0.05) on 1 Sep 2011, 3 Oct 2011 and 6 Dec 2011, where pH was greater (p < 0.05) under the 400N fertiliser rates than the 200N and 0N fertiliser rates. These effects all occurred within 4 days of split fertiliser applications. There was no interaction between the urine and fertiliser treatments on the soil surface pH.

3.3.4 Pasture yield and N uptake

3.3.4.1 Dry matter yield

The first pasture harvest was collected on 23 Mar 2011. From this time to 29 Apr 2011 pasture DM decreased in all treatments, even though fertiliser applications occurred (Figure 3.21a). There were clear peaks in the pasture yields in many treatments around 31 May 2011, 29 Sep 2011, 10 Jan 2012, 10 Mar 2012 and 2 July 2012.

Dry matter yields increased in the month following the concurrent autumn urine/fertiliser application (4 May 2011) from 290, 410 and 550 kg DM ha\(^{-1}\) to 1310, 1560 and 1590 kg DM ha\(^{-1}\) in treatments F0UA, F2UA and F4UA, respectively (Figure 3.21a). Pasture
yields from the autumn urine affected treatments were significantly greater (p < 0.001) than those not affected by autumn urine from 31 May 2011 (day 100) to 28 Jul 2011.

In the month following the concurrent spring urine/fertiliser application (31 Aug 2011), DM yields increased from 280, 210 and 370 kg DM ha\(^{-1}\) to 1960, 2110 and 2060 kg DM ha\(^{-1}\) in treatments F0US, F2US and F4US, respectively (Figure 3.21a). There was a notable increase in pasture DM yields in all treatments, but particularly the spring urine affected treatments between 12 Jan 2012 and 15 Mar 2012. From 6 Sep onwards (after spring urine application), pasture yields from the spring urine treatments were greater (p < 0.001) than those from the non-urine treatments. Pasture yields from the spring urine treatments were also greater than those from autumn urine treatments on three occasions: 3 Nov 2011; 5 Dec 2011 and 7 Feb 2012 (p < 0.05). Pasture yields from the autumn urine treatments were only higher than the spring urine treatments prior to 31 Aug 2011 (before spring urine was applied). Pasture yields were larger (p < 0.001) under urine, regardless of application timing, from the first urine application until the end of the experiment.

Prior to the application of urine, pasture yields increased as a result of fertiliser application (p < 0.001), with no difference in yield between the 200N and 400N rates. After the application of urine, the presence of fertiliser continued to result in significantly higher DM yields (p < 0.05) (compared to the absence of fertiliser) at all but seven individual harvests (6 Sep 2011, 28 Sep 2011, 3 Nov 2011, 17 Nov 2011, 12 Jan 2012, 7 Feb 2012 and 13 Mar 2012). Again, there was no difference in yield between the 400N and 200N fertiliser rates.

There was a significant interaction (p < 0.05) between fertiliser and urine on pasture yields on the 31 May 2011, 27 Jun 2011, and 28 Jul 2011, where under autumn applied urine, pasture yields were greater from the 200N and 400N fertiliser rates compared to the 0N fertiliser rate. There was also an interaction between fertiliser and urine on 14 Apr 2012, and 24 Aug 2012, where under spring applied urine, pasture yields were greater from the 200N and 400N fertiliser rates compared to the 0N rate; and on 17 Nov 2011 where pasture yields from the 0N fertiliser rate were greater than from the 200N and 400N rates (Figure 3.21a).

The greatest cumulative yields occurred in the spring urine treatments (p < 0.001) with means of 19600, 24600 and 27600 kg DM ha\(^{-1}\) from the F0US, F2US and F4US treatments, respectively. This was followed by the autumn urine treatments (p < 0.001)
with yields of 15600, 19600 and 22700 kg DM ha\(^{-1}\) from the F0UA, F2UA and F4UA treatments, respectively; then the nil urine treatments with cumulative yields of 8000, 14000 and 18000 kg DM ha\(^{-1}\) from the F0U0, F2U0 and F4U0 treatments, respectively (Figure 3.21b).

The effect of fertiliser was also significant on cumulative pasture yields with the 400N rate (within each urine treatment) resulting in the highest yields (p < 0.001), followed by the 200N, then 0N rates. There was no interaction between the fertiliser and urine treatments on the cumulative pasture N uptake.

![Figure 3.21](image-url)

**Figure 3.21** (a) Temporal pasture DM yields and (b) cumulative pasture DM yields from all treatments, from 21 Feb 2011 to 28 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), n=4.
3.3.4.2 Pasture N content

Over the course of the experiment, the N content tended to generally trend downwards, with the exception of the peaks in N content as a result of the autumn and spring urine application events. From 13 Mar 2012, the pasture N content in all treatments began to trend upwards, reaching a peak on 17 May 2012, before declining again until the experiment ceased (Figure 3.22).

Pasture N content in the fertiliser affected treatments increased (p < 0.001) after the first harvest from around 3% in all treatments to range from 4.3-5%, while the non-fertiliser treatments increased up to 3.5% (Figure 3.23). The presence of fertiliser had a significant positive effect on the pasture N content (p < 0.05) at every harvest except three: 28 Sep 2011, 3 Nov 2011 and 7 Feb 2012. There were differences (p < 0.05) between the fertiliser rates under nil urine, with the highest N contents observed in the 400N treatments, followed by 200N, then 0N. However, in the urine treatments, the differences between the fertiliser rates were not significant.

Following autumn urine application, pasture N content increased to a peak of 5.1, 4.8 and 4.7% in the F0UA, F2UA and F4UA treatments, respectively. Following spring urine application, pasture N content increased to a peak of 5.5, 5.3 and 5.3% in the F0US, F2US and F4US treatments, respectively (Figure 3.22). In both cases, the pasture N contents at these peaks were greater than the pasture N contents in all other treatments (p < 0.001). After urine application, the pasture N contents in the urine treatments were significantly greater (p < 0.001) than those in the non-urine treatments for substantial periods of the experiment.

There was also a significant interaction of fertiliser rate and autumn urine timing on pasture N on 31 May 2011 and 27 Jun 2011, 6 Sep 2011; and of fertiliser rate with spring urine timing on 6 Sep 2011, 17 Nov 2011 and 5 Dec 2011, where within the urine affected treatments, there was no significant difference in pasture N content between the three fertiliser rates. In the non-urine treatments, pasture N content was greater (p <0.05) under the 400N fertiliser rate compared to the other two rates (31 May 2011; 17 Nov 2011), and, on 6 Sep 2011 pasture N content was greater (p < 0.001) under the 200N rate than the control; or pasture N content was greater (p < 0.05) in the 400N and 200N rates than in the 0N rate (27 Jun 2011, 5 Dec 2011) (Figure 3.22).
3.3.4.3 Pasture N uptake

Temporal patterns in pasture N uptake followed a similar pattern to pasture yields. There were two clear spikes in pasture N uptake, one following autumn urine application and the other following spring urine application. There was also a spike in plant N uptake in all treatments on 12 Jan 2012 (Figure 3.23a).

Following autumn urine application, N uptake in the affected treatments reached peaks of 70, 74 and 76 kg N ha\(^{-1}\) in the F0UA, F2UA and F4UA treatments, respectively. This N uptake was greater (p < 0.001) than that from the non-autumn urine affected treatments until 31 Aug 2011, when the spring urine application occurred. Following spring urine application, pasture N uptake reached peaks of 107, 114 and 108 kg N ha\(^{-1}\) in treatments F0US, F2US and F4US, respectively. This N uptake was greater (p < 0.001) than that from the autumn and nil urine affected treatments until 6 Dec 2011, and then again from 12 Jan 2012 to 7 Feb 2012. Overall, N uptake in the urine treatments (whether autumn or spring applied) was greater (p < 0.001) than N uptake in the non-urine treatments from 31 May 2011 to the end of the experiment (Figure 3.23a).

Under nil urine, there was a fertiliser effect (p < 0.001) on most harvests where N uptake under the 400N fertiliser rate was greater (p < 0.001) than the 200N rate, which was...
greater than the 0N rate. Following autumn urine and spring urine, there was no significant difference between all three fertiliser rates until Mar 2012. After this time, the fertiliser treatment effect (p < 0.001) on N uptake resumed (Figure 3.23a).

Cumulative pasture N uptake followed a similar pattern to cumulative pasture DM yields (Figure 3.23b). The greatest cumulative pasture N uptake occurred in the spring urine affected treatments (p < 0.001) with 700, 900 and 1040 kg N ha\(^{-1}\) from the F0US, F2US and F4US treatments, respectively. This was followed by the autumn urine affected treatments (which were significantly greater (p < 0.001) than the nil urine treatments) where cumulative N uptake was 520, 650 and 780 kg N ha\(^{-1}\) from treatment F0UA, F2UA and F4UA, respectively. Finally, the nil urine treatments had cumulative N uptakes of 210, 420 and 590 kg N ha\(^{-1}\) from the F0U0, F2U0 and F4U0 treatments, respectively (Figure 3.23b).

There was a fertiliser rate effect (p < 0.001), where cumulative N uptake under the 400N rate (within each urine treatment) was greater than that under the 200N rate, which, in turn, was greater than that under the 0N rate (Figure 3.23b). There was no interaction between the urine and fertiliser treatments on cumulative pasture N uptake. However, on specific dates, there was a significant interaction on 27 Jun 2011, 28 Jul 2011 and 6 Sep 2011, where within the autumn urine treatments, the cumulative N uptake was greater (p < 0.05) under the 400N fertiliser rate than the 200N and 0N rates, and greater (p < 0.05) under the 200N rate than the 0N rate (Figure 3.23b). There was no interaction between fertiliser and spring urine on cumulative N uptake.
Figure 3.23 (a) Temporal pasture N uptake and (b) cumulative pasture N uptake from all treatments from 21 Feb 2011 to 28 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + N fertiliser application, other arrows indicate split fertiliser applications. Error bars = LSD (5%), \(n=4\).

### 3.3.4.4 Pasture species composition

At the time the pasture dissection was carried out (2 Jul 2012), the dry matter comprising grass species was > 80% (± 5.9%) in all treatments. The proportion of grass was lower in treatments F0U0, F0UA and F2UA, however, there was no statistical difference in grass content between any of the nine treatments. Consequently, the proportion of weeds was higher in the three treatments with lower grass content at 11.0, 12.5 and 15.5% in the F2UA, F0U0 and F0UA treatments, respectively. In the remaining treatments the weed
content was < 5.5% (Figure 3.24). This greater proportion of weeds in the F2UA, F0U0 and F0UA treatments, likely encroached on the space available for grass in these lysimeters. However, similarly to the grass, there was no statistical difference in the weed content between any of the nine treatments. The mean clover content was comparatively low at < 2.6% in all treatments, and there was no significant difference in clover coverage due to treatments.

**Figure 3.24** Mean proportion of grass, weed and clover species in harvested pasture on 2 Jul 2012. Error bars = (5%), n = 4.

### 3.3.4.5 Temporal fertiliser $^{15}$N recovery in pasture

The pasture harvested from treatments receiving $^{15}$N enriched fertiliser was enriched in $^{15}$N throughout the experiment ranging from 0.6-3.4 atom% (Figure 3.25a). Pasture $^{15}$N enrichment was greater (p < 0.001) from the 400N fertiliser rate, followed by the 200N rate. Pasture $^{15}$N enrichment decreased (p < 0.001) following autumn and spring urine applications.

Fertiliser $^{15}$N recovery in the pasture increased following urine + fertiliser applications and some split fertiliser applications, but these grew progressively smaller over time and the trend declined until 7 Feb 2012 whereupon $^{15}$N recovery increased by ~2% (Figure 3.25).

The greatest recovery of $^{15}$N occurred at the first harvest (23 Mar 2011) and ranged from 11.0-13.5%. There was no significant difference in $^{15}$N recovery between urine or
fertiliser treatments on this date. Recovery of fertiliser $^{15}$N in pasture declined considerably after this, despite another fertiliser $^{15}$N application on 28 Mar 2011.

Following the autumn urine + fertiliser application (31 May 2011), pasture $^{15}$N recovery spiked and the nil urine and spring urine treatments had greater recoveries of fertiliser $^{15}$N than the autumn urine treatments ($p < 0.001$). Following the spring urine + fertiliser application, fertiliser $^{15}$N recovery spiked on the 16 Oct 2011 harvest, and the recovery of $^{15}$N from the nil urine and autumn urine treatments was greater ($p < 0.05$) than that from the spring urine treatments (Figure 3.25).

In general, fertiliser $^{15}$N recovery in pasture appeared greater from treatments that received the 400N fertiliser rate compared to the 200N rate (Figure 3.25). However, there was only one harvest where this was statistically significant ($p < 0.05$) on 5 Dec 2011. There was no interaction between urine and fertiliser treatments on fertiliser $^{15}$N recovery by pasture at any date.
3.3.4.6 Cumulative fertiliser $^{15}$N recovery in pasture

Total recovery of fertiliser $^{15}$N in the pasture ranged from 51-62% (Figure 3.26). There was a urine effect with greater ($p < 0.05$) fertiliser $^{15}$N recovery in the spring urine treatments than the autumn urine treatments.

The greatest cumulative recovery of $^{15}$N by pasture was from the 400N fertiliser treatments with total cumulative recoveries of 62, 59 and 55% from the F4US, F4U0 and F4UA treatments, respectively (Figure 3.26). Cumulative recoveries of $^{15}$N from the
200N fertiliser treatments were 57, 52, and 50% for the F2US, F2U0 and F2UA treatments, respectively (Figure 3.26). Cumulative fertiliser $^{15}$N recovery was greater ($p < 0.05$) in treatment F4US than treatments F2UA and F2U0, and it was also greater ($p < 0.05$) in the F4U0 treatment than the F2UA treatment. Other than these effects, there were no other significant differences in cumulative fertiliser $^{15}$N recovery due to treatment.

**Figure 3.26** Mean cumulative pasture $^{15}$N recovery from all treatments that received $^{15}$N labelled fertiliser from 21 Feb 2011 to 24 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Arrows indicate N fertiliser application; bold arrows indicate urine + N fertiliser application. Error bar = LSD (5%), n = 4.

### 3.3.4.7 N content and fertiliser $^{15}$N recovery in roots and stubble

The root DM yield ranged from 15-39 kg DM ha$^{-1}$. The root DM yield from the F2UA treatment was greater ($p < 0.001$) than that of all other treatments. Apart from this, treatments did not influence DM yields. Stubble, DM yield ranged from 28 (F4U0) to 48 (F0US) kg DM ha$^{-1}$ but did not differ with treatment (Figure 3.27).
Figure 3.27  Mean dry matter content in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4.

The mean N content ranged from 1.6-1.9% in the roots and 1.6-1.8% in the stubble (Figure 3.28) with no effect of treatment.

Figure 3.28  Mean N content (%) in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4.

Root N uptake ranged from 0.25-0.76 kg N ha⁻¹ (Figure 3.29). Root N uptake in the F2UA treatment was greater (p < 0.001) than all other treatments with no differences
between the other treatments. Stubble, N uptake ranged from 0.47 (F4U0) to 0.83 (F2UA) kg N ha\(^{-1}\) with no difference due to treatment (Figure 3.29).

![Figure 3.29 Mean N uptake in the roots and stubble for each treatment at the end of the experiment. LSD (5%) is shown for (a) roots and (b) stubble, n = 4.](image)

Recovery of fertiliser \(^{15}\)N ranged from 0.010 (F4UA) to 0.031\% (F2UA) in the roots (Figure 3.30) and was greater (p < 0.001) in treatment F2UA, but there was no significant treatment effect.

The recovery of fertiliser \(^{15}\)N ranged from 0.016 (F4U0) to 0.039\% (F2U0) in the stubble, and was greater (p < 0.001) in treatments F2U0 and F2UA, than in the other treatments (F4U0, F2US and F4US), with the exception of the F4UA treatment (Figure 3.30).

There was a significant fertiliser effect on the recovery of \(^{15}\)N in both the roots (p = 0.05) and stubble (p < 0.05), where the \(^{15}\)N recovered in both roots and stubble was greater from treatments that received the 200N fertiliser rate, compared to those that received the 400N rate (Figure 3.30). There was no effect of urine on the recovery of \(^{15}\)N in the roots or stubble at the end of the experiment, nor was there any interactive effect between urine and fertiliser.
3.3.5 Soil N

3.3.5.1 Bulk density

The bulk density of the Horotiu silt loam from the lysimeter collection site at the beginning of the experiment is shown below (standard error of the mean indicated in parentheses, n = 2). Soil bulk density decreased with increasing depth until 40 cm, after which it remained at around 0.80 g cm\(^{-3}\).

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Mean bulk density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0.87 (0.015)</td>
</tr>
<tr>
<td>10-20</td>
<td>0.84 (0.012)</td>
</tr>
<tr>
<td>20-40</td>
<td>0.79 (0.033)</td>
</tr>
<tr>
<td>40-60</td>
<td>0.80 (0.011)</td>
</tr>
<tr>
<td>60-70</td>
<td>0.80 (0.011)</td>
</tr>
</tbody>
</table>

3.3.5.2 Gravimetric soil moisture at end of trial (27 Aug 12)

Soil moisture tended to decrease with increasing depth and ranged between 0.65-0.79 g H\(_2\)O g\(^{-1}\) oven dry soil at the surface depth (0 to 7.5 cm) and 0.47-0.61 g H\(_2\)O g\(^{-1}\) oven dry
soil at the deepest depth (30-70 cm) (Figure 3.31). Soil moisture was not significantly affected by urine treatment, nor was it affected by fertiliser rate. There was no interactive urine and fertiliser rate effect on soil moisture at any depth.

Figure 3.31 Mean gravimetric soil moisture with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4.

3.3.5.3 Inorganic soil N (NH\(_4^+\)-N and NO\(_3^-\)-N)

At the end of the experiment, soil NH\(_4^+\)-N concentrations and the range between treatment means tended to decrease with increasing depth, with concentrations ranging from 1.7-3.3 and 0.8-1.3 \(\mu g\) NH\(_4^+\)-N g\(^{-1}\) dry soil in the top 5 cm and bottom 30-70 cm of soil, respectively (Figure 3.32). In the top 5 cm of soil the urine treatments had greater NH\(_4^+\)-N concentrations than the non-urine treatments (p < 0.001). In the top 10 cm of soil there was a significant fertiliser effect, whereby the treatments that received fertiliser had greater concentrations than those that did not (p < 0.05). However, the rate of fertiliser did not significantly affect the soil NH\(_4^+\)-N concentrations. Below 10 cm depth, there was no significant treatment effect on NH\(_4^+\)-N concentrations.
At the end of the experiment, soil NO₃⁻-N concentrations decreased with depth (p < 0.001), and concentrations ranged from 1.5-2.5 and < 0-0.4 µg NO₃⁻-N g⁻¹ dry soil in the top 5 cm and bottom 30-70 cm soil, respectively (Figure 3.33). In the top 5 cm of soil, NO₃⁻-N concentrations in the urine treatments were greater than those from the non-urine treatments (p < 0.05). The timing of the urine application (autumn vs spring) did not affect the soil NO₃⁻-N concentrations. The presence of fertiliser had an effect (p < 0.05) on soil NO₃⁻-N concentrations at the 5-15 cm depth in the autumn and spring urine treatments, where the highest soil NO₃⁻-N concentrations occurred under nil fertiliser followed by the 400N and 200N fertiliser rates, respectively. There was no interaction between the urine and fertiliser treatments on soil NO₃⁻-N at the end of the experiment.
Figure 3.33  Mean soil NO$_3^-$-N concentration with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4.

Total inorganic N concentrations across all depths ranged from 7.5 (F2UA) to 10.6 (F4U0) kg N ha$^{-1}$, and were dominated by the NH$_4^+$-N fraction (Figure 3.34). There was no significant treatment effect (urine or fertiliser), nor any interactive effect between the treatments on the total soil inorganic N at the end of the experiment (Figure 3.34).

Figure 3.34  Total soil inorganic N comprising soil NH$_4^+$-N and NO$_3^-$-N at the end of the trial summed over the 0 to 70 cm depth. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; U0, UA and US denote nil, autumn and spring urine applications, respectively. LSD (5%) is shown for (a) NH$_4^+$-N and (b) NO$_3^-$-N, n = 4.
3.3.5.4 Total soil N

Total soil N was determined for each depth (Figure 3.35), and the total soil N for each lysimeter was calculated (kg ha\(^{-1}\)) (Figure 3.36). Total soil N (%) was greatest in the top 5 cm, ranging from 0.7-0.9% and decreased with increasing depth, ranging from 0.15-0.25% at 30–70 cm. Soil total N in the 0-5 cm depth was greater (p < 0.05) in the treatments that received fertiliser, than those that did not (with the exception of the F0UA treatment). This effect was not evident in any of the remaining depth layers. There was no interaction of the urine and fertiliser treatments on total soil N at any depth.

Total soil N (kg ha\(^{-1}\)) in the lysimeter profiles ranged from 18700-22000 kg N ha\(^{-1}\) (Figure 3.36). The F4U0 and F2US treatments had more total N (p < 0.05), than the F2U0, F4UA and F0US treatments, and with the exception of these, there were no significant differences in total soil N between treatments. There was a urine and fertiliser interaction (p < 0.05) where under nil urine, total soil N tended to increase with increasing fertiliser rate while in the autumn urine and spring urine treatments there was no trend with fertiliser rate whatsoever.

![Figure 3.35](image)

**Figure 3.35** Mean total soil N (%) with depth at the end of the trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4.
Figure 3.36  Mean total soil N (kg ha\(^{-1}\)) in lysimeters at the end of the trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4.

3.3.5.5 Microbial biomass N

Soil microbial biomass N at the end of the experiment decreased with increasing soil depth. Microbial biomass N concentrations ranged from 204-262 µg N g\(^{-1}\) soil in the top 5 cm and from 10-34 µg N g\(^{-1}\) soil in the 30-70 cm depth (Figure 3.37). There were no urine or fertiliser effects on soil microbial biomass N, nor any interaction between urine and fertiliser.

Figure 3.37  Mean soil microbial biomass N with depth at end of trial. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); U0, UA and US denote nil, autumn and spring urine applications, respectively. Error bars = LSD (5%), n = 4.
3.3.5.6  **Fertiliser $^{15}$N recovery in soil**

Recovery of $^{15}$N in the soil (inorganic-N + organic-N) was greater in the top 5 cm of soil ($p < 0.001$), ranging from 10.3-14.1% recovery. At 5-10 cm depth, soil $^{15}$N recovery reduced considerably, ranging from 1.01-3.58%. Below 10 cm depth, soil $^{15}$N recovery remained ≤ 2.38% in all treatments (Table 3.7). Total recovery of $^{15}$N tended to be higher in the treatments that received nil urine, compared to those that received autumn and spring urine, but this was not significant. There was no significant treatment effect or interaction on the soil $^{15}$N recovery at any depth (Figure 3.38).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>F2U0</th>
<th>F2UA</th>
<th>F2US</th>
<th>F4U0</th>
<th>F4UA</th>
<th>F4US</th>
<th>LSD (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>14.1</td>
<td>10.6</td>
<td>12.3</td>
<td>10.6</td>
<td>10.3</td>
<td>11.8</td>
<td>2.79</td>
</tr>
<tr>
<td>5-10</td>
<td>3.58</td>
<td>2.23</td>
<td>3.01</td>
<td>3.21</td>
<td>3.04</td>
<td>2.42</td>
<td>1.01</td>
</tr>
<tr>
<td>10-15</td>
<td>0.91</td>
<td>0.80</td>
<td>1.17</td>
<td>1.73</td>
<td>0.94</td>
<td>0.89</td>
<td>1.05</td>
</tr>
<tr>
<td>15-30</td>
<td>1.33</td>
<td>1.34</td>
<td>1.89</td>
<td>1.81</td>
<td>1.91</td>
<td>1.09</td>
<td>1.41</td>
</tr>
<tr>
<td>30-70</td>
<td>1.65</td>
<td>1.55</td>
<td>1.63</td>
<td>2.38</td>
<td>1.15</td>
<td>0.84</td>
<td>2.21</td>
</tr>
</tbody>
</table>

**Table 3.7  Fertiliser $^{15}$N recovered in soil (%) at each depth at the end of the trial.**

**Figure 3.38**  Fertiliser $^{15}$N recovered in soil. Values represent the $^{15}$N recovery summed over all depths.
3.3.6 Mass Balance

3.3.6.1 Fertiliser $^{15}$N recovery balance

Total fertiliser recovery as $^{15}$N at the end of the experiment (27 Aug 2012) ranged from 68.4 (F2UA) to 81.4% (F4US), leaving 31.6 to 18.6% of the $^{15}$N unaccounted for (Table 3.8). The pasture fraction contributed the highest fertiliser $^{15}$N recovery, ranging from 50.5 to 62.2%, followed by the total soil fraction, ranging from 16.5-21.6%, then the leached inorganic N fraction, ranging from 0.03-2.17%. The remaining fractions (N$_2$O emissions, roots and stubble) contributed negligible amounts, <0.1% to the total $^{15}$N recovery.

The application of fertiliser had an effect on total $^{15}$N recovery ($p = 0.05$) with greater total recovery in the treatments that received the 400N rate than the 200N rate. There was also a urine effect ($p < 0.05$) where the total $^{15}$N recovery was greatest in the spring urine treatments, followed by the nil urine treatments, and was lowest in the autumn urine treatments. There was no interaction between the treatments on total $^{15}$N recovery.
### Table 3.8 Mass balance of fertiliser $^{15}$N recovery from lysimeters at the end of the experiment (27 Aug 2012).

<table>
<thead>
<tr>
<th>Variant</th>
<th>Variations</th>
<th>Fert Urine Inter-</th>
<th>Significance of variation$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatments (% recovered as $^{15}$N)</td>
<td>LSD (5%)$^1$</td>
<td>Fert</td>
</tr>
<tr>
<td></td>
<td>F2U0</td>
<td>F2UA</td>
<td>F2US</td>
</tr>
<tr>
<td>Pasture</td>
<td>52.4 (ab)</td>
<td>50.5 (a)</td>
<td>57.2 (abc)</td>
</tr>
<tr>
<td>Total soil N</td>
<td>21.6 (a)</td>
<td>16.5 (a)</td>
<td>20.0 (a)</td>
</tr>
<tr>
<td>$N_2O$</td>
<td>0.02 (a)</td>
<td>0.04 (ac)</td>
<td>0.02 (a)</td>
</tr>
<tr>
<td>Leached inorg N</td>
<td>0.03 (a)</td>
<td>1.35 (a)</td>
<td>0.73 (a)</td>
</tr>
<tr>
<td>Roots</td>
<td>0.05 (a)</td>
<td>0.07 (b)</td>
<td>0.04 (a)</td>
</tr>
<tr>
<td>Stubble</td>
<td>0.04 (a)</td>
<td>0.04 (b)</td>
<td>0.02 (b)</td>
</tr>
<tr>
<td>Total $^{15}$N</td>
<td>74.1 (ac)</td>
<td>68.4 (a)</td>
<td>78.0 (bc)</td>
</tr>
</tbody>
</table>

$^{15}$N not accounted for$^3$

|                | 25.9 | 31.6 | 22.0 | 20.6 | 25.6 | 18.6 |

---

1. Different letters indicate a significant difference between treatments at the 5% (p < 0.05) level.
2. Significance of variation presented as: * P<0.05; ** P<0.01; *** P<0.001, NS Not significant P>0.05).
3. $^{15}$N not accounted for (%) = 100% - total $^{15}$N recovery%.

### 3.3.6.2 Apparent N balance

The apparent mass recovery of N ranged from 218 kg ha$^{-1}$ in the control, to 1130 in the F4UA treatment (Table 3.9). There was a highly significant fertiliser effect where, within each urine treatment, the mass recovery was greatest under the 400N fertiliser rate, followed by the 200N rate, then the 0N rate (p < 0.001). There was also a urine effect where the mass recovery of N was greatest under autumn applied urine, followed by spring applied urine, then nil urine (p < 0.001).

The apparent recovery of N as a percentage of the total N applied (urinary and fertiliser N) ranged from 65-89% (Table 3.9). The pasture fraction contributed the greatest apparent N recovery, ranging from 40-68%. This was followed by the leached inorganic N fraction, ranging from 1-40%, and the leached DON fraction, ranging from 0.3-10% recovery. The contribution of N$_2$O emissions to the apparent N recovery was <0.6%
across all treatments, and the contribution from the roots and stubble was even lower, at <0.1% across all treatments.

Apparent % recovery increased with decreasing fertiliser rate (p < 0.05), (i.e. the apparent % recovery was greater under the 0N rate, followed by the 200N rate, then the 400N rate). There was a stronger urine effect where the apparent % N recovery was greatest (p <0.01) in the autumn urine treatments, followed by the nil urine treatments, then the spring urine treatments. There was no interaction between the fertiliser and urine treatments on the mass recovery of N as a % of the total N applied.

Table 3.9 Apparent Mass balance of treatment associated N recovery (urine and fertiliser) from lysimeters at the end of the experiment (27 Aug 2012).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total N applied (kg ha⁻¹)</th>
<th>Total recovery (kg ha⁻¹)</th>
<th>Total recovery (%)</th>
<th>Pasture (%)</th>
<th>Roots &amp; Stubble (%)</th>
<th>N₂O emissions (%)</th>
<th>Leached inorg N³ (%)</th>
<th>Leached DON³ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0U0</td>
<td>0</td>
<td>218 (a)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02(ab)</td>
<td>0.57 (a)</td>
<td>40.0 (a)</td>
</tr>
<tr>
<td>F0UA</td>
<td>800</td>
<td>928 (b)</td>
<td>88.8 (a)</td>
<td>37.5 (a)</td>
<td>0.04(ab)</td>
<td>0.42 (ac)</td>
<td>2.54 (bd)</td>
<td>0.57 (b)</td>
</tr>
<tr>
<td>F0US</td>
<td>800</td>
<td>742 (c)</td>
<td>65.5 (b)</td>
<td>60.6 (b)</td>
<td>0.09 (a)</td>
<td>0.23 (bd)</td>
<td>0.97 (b)</td>
<td>0.46 (b)</td>
</tr>
<tr>
<td>F2U0</td>
<td>300</td>
<td>428 (d)</td>
<td>70.0 (b)</td>
<td>68.1 (b)</td>
<td>0.07(ab)</td>
<td>0.49 (a)</td>
<td>26.0 (c)</td>
<td>5.87 (c)</td>
</tr>
<tr>
<td>F2UA</td>
<td>1100</td>
<td>1008 (ef)</td>
<td>71.8 (b)</td>
<td>39.3 (a)</td>
<td>0.17 (a)</td>
<td>0.49 (a)</td>
<td>26.0 (c)</td>
<td>5.87 (c)</td>
</tr>
<tr>
<td>F2US</td>
<td>1100</td>
<td>940 (bf)</td>
<td>65.7 (b)</td>
<td>62.4 (b)</td>
<td>0.01 (b)</td>
<td>0.27 (bc)</td>
<td>2.12 (bd)</td>
<td>0.31 (b)</td>
</tr>
<tr>
<td>F4U0</td>
<td>600</td>
<td>605 (g)</td>
<td>64.5 (b)</td>
<td>62.1 (b)</td>
<td>0.01 (b)</td>
<td>0.16 (b)</td>
<td>1.46 (bd)</td>
<td>0.32 (b)</td>
</tr>
<tr>
<td>F4UA</td>
<td>1400</td>
<td>1260 (h)</td>
<td>74.5 (b)</td>
<td>40.4 (a)</td>
<td>0.01 (b)</td>
<td>0.41 (acd)</td>
<td>26.9 (c)</td>
<td>6.19 (c)</td>
</tr>
<tr>
<td>F4US</td>
<td>1400</td>
<td>1130 (i)</td>
<td>65.1 (b)</td>
<td>58.5 (b)</td>
<td>0.02 (b)</td>
<td>0.25(bcd)</td>
<td>4.49 (d)</td>
<td>0.89 (b)</td>
</tr>
<tr>
<td>LSD (5%)³</td>
<td>77.3</td>
<td>11.4</td>
<td>12.0</td>
<td>0.07</td>
<td>0.18</td>
<td>3.10³</td>
<td>0.77³</td>
<td></td>
</tr>
</tbody>
</table>

Significance of variation²

| Fertiliser | *** | *  | NS  | NS  | **  | *  | *  | *  |
| Urine      | *** | ** | *** | NS  | *** | *** | *** | *** |
| Interaction| NS  | NS | NS  | NS  | NS  | NS | NS | NS |

1 Different letters indicate a significant difference between treatments at the 5% (p < 0.05) level.
2 Significance of variation presented as: * P<0.05; ** P<0.01; *** P<0.001, NS Not significant P>0.05).
3 Mean leached inorganic N and leached DON values presented are the back-transformed square root values; error for these variants is the average SED (5%).
3.4 Discussion

3.4.1 Leachate

Drainage:

There was little variation in drainage from the lysimeters until July 2011, where the urine and fertiliser treatment effects began, suggesting there were no edge flow concerns or impediments to flow in the lysimeters. The urine and fertiliser treatment effects observed were due to increased pasture DM yield and consequently, increased uptake of soil water by the pasture as a result of the N added to the system in the treatment affected lysimeters (Figure 3.21b). The greatest differences in drainage occurred during the spring months, where pasture growth was also at its greatest.

Leached $NH_4^+\cdot$N:

Leached $NH_4^+\cdot$N throughout the experiment was negligible with the exception of the peaks observed in the F2UA treatment on 23 May 2011, and the F0US treatment on 4 Oct 2011 (Figure 3.8). These were, in both cases, the result of high $NH_4^+\cdot$N concentrations in the leachate in only one of the four replicates for that treatment, indicating its appearance was a result of transport due to preferential flow after a drainage event. Preferential flow has also been observed under urine patches in other studies (Williams et al., 1990b; Clough et al., 1996). Even the urine application itself may result in temporary ponding on the lysimeter surface, which can initiate preferential flow (Williams et al., 1990b; Williams and Haynes, 1994). Prior to 23 May 2011 there were many small rainfall events (< 10 mm) that contributed to drainage, and on 3 Oct 2011, there was a 24 mm rainfall event, that followed many small rainfall events between the 10 and 27 Sept 2011 Oct, resulting in drainage.

Leached $NO_3^-\cdot$N:

The 3-month lag between autumn urine application and peak $NO_3^-\cdot$N concentrations in the drainage water is a function of the rate of nitrification and the amount of drainage (rainfall less evapotranspiration and soil water storage). Previous studies on urine-N fate in temperate environments have recorded similar lag periods of 1 to 3 months (Clough et al., 1996; Silva et al., 1999; Di and Cameron, 2007). The peak concentration of $NO_3^-\cdot$N under autumn applied urine in other pasture based lysimeter studies ranged from 50-225 mg N L$^{-1}$ (Di et al., 2002; Decau et al., 2003; Silva et al., 2005; Di and Cameron, 2007).
The peak NO$_3^-$-N concentrations observed under autumn urine treatments of up to 205 mg NO$_3^-$-N L$^{-1}$ in this study were within the higher end of this range.

The time lag in the appearance of peak NO$_3^-$-N concentrations following spring urine application increased as fertiliser rate increased because additional fertiliser promoted pasture growth (Figure 3.21) which in turn progressively exacerbated water loss via evapotranspiration (Figure 3.6) and delayed the onset of NO$_3^-$-N leaching. The peak concentration of NO$_3^-$-N was similar to previously measured NO$_3^-$-N losses under spring urine (Di and Cameron, 2002a; Decau et al., 2004). The higher peak NO$_3^-$-N concentrations with increasing fertiliser rate following spring urine application were a consequence of increasing N availability in soil and thus greater potential for leaching.

Greater NO$_3^-$-N leaching from autumn applied urine compared with spring applied urine was expected, due to increased rainfall and soil drainage, decreased evaporative loss, decreasing temperatures and decreased plant uptake during the autumn-winter period. Other studies have also reported significant seasonal variability in NO$_3^-$-N leaching losses from urine affected soils, with greater losses in the wetter cooler seasons compared with warmer drier seasons (Di and Cameron, 2002b, a; Decau et al., 2003; Cameron et al., 2013).

In the case of both autumn and spring applied urine, peak NO$_3^-$-N concentrations occurred before one pore volume of drainage had occurred. This is further indication that nitrate transport down the soil profile was not entirely a result of uniform displacement, but also preferential flow through soil macropores.

Total cumulative NO$_3^-$-N leaching losses following the autumn urine application were higher (300-390 kg NO$_3^-$-N ha$^{-1}$) than those following the spring urine application, (11-50 kg NO$_3^-$-N ha$^{-1}$) and nil urine (0.5-7.5 kg NO$_3^-$-N ha$^{-1}$) treatments. Again this is a reflection of the lack of treatment effects on pasture N uptake following the autumn urine application (Figure 3.23) compared to following the spring urine application. Given that plant and microbial demand for N was lower at this time, due to lower soil and air temperatures (Figure 3.6) the N uptake was reduced and the surplus N was thus nitrified and leached from the system.

A further factor in the enhanced leaching of NO$_3^-$-N from under autumn urine was the amount of rainfall following the autumn application of urine compared to the spring.
After autumn application the equivalent of 1 pore volume of drainage occurred within 180 days, while following the spring application this took 330 days due to reduced rainfall over this season.

The clear fertiliser effect on NO$_3^-$-N leached in the autumn and spring urine treatments and the marginal fertiliser effect on the nil urine treatments suggests that fertiliser applications in combination with a urine patch enhance NO$_3^-$-N leaching losses. However, oddly, the NO$_3^-$-N losses under the autumn urine treatments were greater from the F4UA treatment followed by the F0UA, then F2UA treatments. Prior to autumn urine application, the lysimeters had received 2 split applications of fertiliser (21 Feb 2011 and 28 Mar 2011), and prior to this, the lysimeter collection site had not received fertiliser for over a year. When plant roots encounter a zone of nutrients, they tend to proliferate within it by deploying primary and secondary lateral roots, and expand their capacity for nutrient uptake (Figure 3.39) (Drew, 1975; Hodge, 2004). It is thought that these root system responses, also often termed “morphological plasticity”, are a natural coping mechanism for plants to overcome the heterogeneous supply of nutrients in the soil and effectively compete underground for nutrients (Hutchings and de Kroon, 1994; Casper and Jackson, 1997; Hodge, 2004).

![Figure 3.39 Root proliferation of barley in localised supplies of phosphate, nitrate, ammonium and potassium. Control (HHH) received a complete nutrient solution to the entire root system. Letters denote L, low; and H, high, referring to the nutrient concentrations available in the upper, middle and lower sections of the root mass, the middle section being between the two lines. Data from Drew (1975); adapted by Hodge (2004).](image)

Therefore, it is highly likely that root proliferation occurred following the initial fertiliser applications in the treatments which received fertiliser prior to autumn urine application, creating a higher capacity for the pasture in those treatments to take up more fertiliser and...
urinary N, compared to the treatments that received no fertiliser. This may explain why more NO$_3^-$-N was lost from the F0UA than F2UA treatments, and in the case of F4UA, although root proliferation and additional plant uptake occurred, the NO$_3^-$-N supply exceeded plant requirements that much more, generating the highest losses.

Cumulative recovery of fertiliser $^{15}$N as NO$_3^-$-N decreased over time in all except the spring urine treatments (Figure 3.12). This occurred because the ongoing split applications of $^{15}$N enriched fertiliser increased the denominator of Equation 3.2, while the cumulative fertiliser $^{15}$N recovery in the leachate did not increase proportionally. Thus less fertiliser N appeared in the leachate as NO$_3^-$-N as time progressed. It should be noted, however, that the fertiliser $^{15}$N recovered as leached NO$_3^-$-$^{15}$N between any two dates is not necessarily derived from the $^{15}$N enriched fertiliser application at the first of these dates since NO$_3^-$ formation and transport is a function of nitrification rates, drainage, and other N competing processes such as plant uptake. Fertiliser $^{15}$N recovery from the spring urine treatments was negligible up until the 2012 drainage season, possibly because the exceptionally high summer rainfall in the Dec 2011-Jan 2012 period induced NO$_3^-$-N transport below the root zone and leaching.

Even in the presence of urine, the cumulative recovery of fertiliser $^{15}$N as inorganic NO$_3^-$-N was $<$2.2%, indicating that although up to 390 kg NO$_3^-$-N ha$^{-1}$ was lost from the system, only a small fraction of this was fertiliser derived NO$_3^-$-N (there was negligible fertiliser $^{15}$N recovered as inorganic NH$_4^+$-N). The results of this study show that when urea fertiliser is applied on its own at regular small rates (up to 50 kg N ha$^{-1}$ per application) its contribution to NO$_3^-$ leaching is negligible ($<$0.2%). When there is a urine patch present the loss of fertiliser N increases, but it is still negligible when compared to the urine induced N loss in leachate. Previous studies have investigated leaching losses under urine patches amended with fertiliser, and while some of these have used $^{15}$N enrichment of the urine to ascertain the significance urine patches play in NO$_3^-$-N leaching (e.g. Decau et al. (2004), Silva et al. (2005), Leterme et al. (2003), and Silva et al. (1999)), no studies, to date, have specifically partitioned the fertiliser component of the urine patch-fertiliser combination to determine its effect on NO$_3^-$-N leaching. Dowdell and Webster, (1980) reported fertiliser $^{15}$N recovery in leachate from a free-draining sandy loam soil (without the presence of a urine patch) of 1-5%; and Barraclough et al. (1984) reported fertiliser $^{15}$N recoveries of 0.14% and 3.1% from fertiliser rates of 250 and 500 kg N ha$^{-1}$ yr$^{-1}$, applied as 3 or 4 applications over three
years. The fertiliser $^{15}$N recoveries obtained in the current study are within the same range as these earlier studies. Other studies have recovered more fertiliser N, for example Di and Cameron (2002a) applied 200 kg N ha$^{-1}$ urea in 4 split applications (April, August, November and February) and recovered 6.2% in the leachate after 1 year (although this study did not use $^{15}$N enriched fertiliser) and van der Kruijs et al. (1988) recovered 22-29% fertiliser $^{15}$N in leachate from cropped lysimeters that received 138 kg N ha$^{-1}$ $^{15}$N enriched urea in Nigeria. Leaching of N inputs is also related to plant and microbial N demands which in turn are a function of plant species, soil fertility and climate, so some caution is needed when comparing studies.

Low fertiliser $^{15}$N recovery as NO$_3^{-}$-N in the current study was due to pasture uptake or microbial uptake of the fertiliser N before it could be leached beyond the root zone. This is supported by the fact that the pasture $^{15}$N recovery ranged from 50-62% (Figure 3.26) and soil $^{15}$N recovery ranged from 16-22% of fertiliser $^{15}$N (Figure 3.38); both components significantly greater than the recoveries as NO$_3^{-}$-N in the leachate.

Although the cumulative recovery of fertiliser $^{15}$N as leached NO$_3^{-}$-N was low (<2.2%) in the autumn and spring urine treatments, the difference in the mean total cumulative NO$_3^{-}$-N leached between the F4UA and F0UA treatments was 55 kg NO$_3^{-}$-N ha$^{-1}$ and between the F4US and F0US treatments was 47 kg NO$_3^{-}$-N ha$^{-1}$. This suggests that the application of fertiliser at the 400 kg N ha$^{-1}$ yr$^{-1}$ rate induced an additional 55 and 47 kg NO$_3^{-}$-N leached under autumn and spring applied urine, respectively. It is possible that the majority of the fertiliser N was taken up by plants and/or microbes, leaving little capacity for uptake of urinary N, thereby making more of it available for leaching (compared to the urine only scenario). However, what is conflicting here is the fact that in the autumn urine treatments, NO$_3^{-}$-N leached from the F0U0 treatment was 45 kg NO$_3^{-}$-N ha$^{-1}$ more than from the F2UA treatment, and under spring urine, there was no difference in NO$_3^{-}$-N leached between the F0US and F2US treatments, suggesting that in fact fertiliser applied at this rate either decreases or does not affect the total NO$_3^{-}$-N leaching. Perhaps there is a ‘tipping point’, in other words a fertiliser rate that, under the current experimental conditions, lies somewhere between 200 and 400 kg N ha$^{-1}$ yr$^{-1}$ where the application of that fertiliser begins to induce additional urine-associated leaching? Without a greater range of fertiliser rates tested, and furthermore, $^{15}$N labelling of the urine, this suggestion remains speculative. Further research is therefore required, that focusses on a greater
range of fertiliser rates applied concurrently with urine, to determine at what point, the application of fertiliser induces a greater leaching risk of urinary N.

Dissolved Organic Nitrogen:

Most pasture leaching studies, and in fact most leaching studies, have overlooked the measurement of DON. Until recently, it has not been considered a significant pathway for N loss from agricultural systems (van Kessel et al., 2009). In this current study, DON collected in the leachate from the autumn urine treatments (64-87 kg DON ha\(^{-1}\)) and spring urine treatments (2.0-12 kg DON ha\(^{-1}\)) was ca. 22% of the NO\(_3^-\) collected (Figure 3.14). Unfortunately, fertiliser \(^{15}\)N recovery was not obtained from the leachate DON pool, but considering the relative amount leached following the autumn urine application, this should be considered as an important area for further research. In a review by van Kessel et al. (2009) the average amount of DON lost in leachate from 16 studies, over a range of agricultural land uses, was about one-third the leaching losses observed for NO\(_3^-\) -N, slightly higher than what was observed here.

In the same summary, van Kessel et al. (2009), reported leached DON from pasture, grassland, and grass/clover systems ranged from 0.3-127 kg DON ha\(^{-1}\) with an average of 12.7 kg DON ha\(^{-1}\), and most values below 10 kg DON ha\(^{-1}\). Only one of these studies had measured DON losses after the application of urine (Wachendorf et al., 2005), who measured 127 kg DON in the leachate. Although this was much higher than the leached DON measured in this study, the urine application rate was higher, at 1030 kg N ha\(^{-1}\), and the lysimeters consisted of sand with an intact pasture sod and were thus very free draining. Smaller leaching losses of DON have been measured in other studies from grazed pastures, e.g. Hoogendorn et al. (2011) measured 44 kg DON ha\(^{-1}\), however, this was a grazed field trial on a strongly leached pumice soil, and furthermore, the leached DON was measured using porous ceramic cup samplers, so these results are not directly comparable to the current lysimeter study where urine was directly applied and the leachate measured from the same area. On the other hand, a lysimeter study by Shepherd et al. (2010) reported 40 kg DON ha\(^{-1}\) leached over a period of 8 months following urine application in late winter (early August) from an identical soil type, the Horotiu silt loam. The urine rate applied was 500 kg N ha\(^{-1}\), 300 kg N ha\(^{-1}\) less than that applied in the current study, and the experiment ran from early August to April, so did not cover the key winter drainage period, yet the DON leached was more than double that observed under
spring urine in the current study. The disparity is most likely due to differences in climatic and rainfall conditions between the two studies. Leached DON was calculated in a range of soil types by Ghani et al. (2010), who multiplied measured amounts of leached organic N from lysimeters and up-scaled them using annual drainage values modelled by Overseer®. This study included three allophanic soils (the Horotiu silt loam is also an allophanic soil) from which the DON losses reported ranged from 29-43 kg DON ha⁻¹. However, urine was not applied to these lysimeters, rather, they were collected from previously grazed sites, and leaching of soil solution was enforced by applying large amounts of water, rather than measuring natural drainage; therefore, the results are not comparable.

Dissolved organic N that is measured in the leachate is that which has remained in the aqueous phase (soil solution), rather than being adsorbed into the soil matrix. The partitioning of organic material between the aquatic and solid phase is dependent on the properties of the organic material itself, the microbial population, and the mineralogical and chemical properties of the soil. Soils with higher proportions of clay have a greater surface area (i.e. layers Al and Fe oxides), therefore the sorption of DON and other soil solutes increases with increasing soil clay content (Bolan et al., 2011). The Horotiu soil is free draining and has a low clay content, which may limit its ability to adsorb DON and may account for the large amount of DON measured in the leachate.

The deposition of urine onto pasture results in a temporary increase in soil pH (Figure 3.20), following the hydrolysis of urea. This results in enhanced solubilisation of soil organic material, and thereby increases the DON content in soil solution (van Kessel et al., 2009; Bolan et al., 2011). This is illustrated by the negligible amounts of DON leached in the nil urine treatments compared to the autumn and spring urine treatments (Figure 3.14). Furthermore, high concentrations of salt introduced to the soil by urine application may have induced osmotic stress and resulted in the release of N through lysis of microbial biomass, thus favouring DON leaching in the urine treatments (Wachendorf et al., 2005). The large difference in DON leached between the autumn urine and spring urine treatments is largely due to the winter drainage that occurred soon after autumn urine application, rapidly leaching the solubilised DON. Additionally, organic compounds embodied in the urine including creatin and creatinin and allantoin, which make up 1-10 and 2-12% of the total N constituents of urine (Whitehead, 1995) could have also contributed to some of the leached DON load in the autumn urine treatments.
Conversely, in the spring urine treatments, drainage did not occur until ca. 4 months after urine application. During this time, warmer air and soil temperatures and longer daylight hours resulted in increased microbial and pasture uptake of the solubilised soil N, and re-adsorption of DON onto soil exchange sites would have occurred, thus resulting in the considerably lower DON leached from the spring urine treatments during the drainage events that occurred in Dec 2011 – Jan 2012.

Similarly to the cumulative NO₃⁻-N leached in the autumn urine treatments, the cumulative DON leached was greatest in the F4UA and F0UA treatments, and lower in the F2UA treatment. This can be attributed to the same theory described earlier for NO₃⁻-N; that the early fertiliser applications caused root proliferation and greater N uptake capacity in the F2UA and F4UA treatments, and when autumn urine was applied, pasture in these treatments was able to assimilate more N than in the F0UA treatment. Although the pasture in treatment F4UA would have increased capacity to take up more N, the overall N applied was still greater than in the F2UA treatment, and hence so was the DON leached. In fact interestingly, the difference in DON leached between treatments F2UA and F4UA was 24 kg DON ha⁻¹ and the difference in one split fertiliser application between the same two treatments was 25 kg N ha⁻¹.

3.4.2 Nitrous oxide

Nitrous oxide emissions as a proportion of applied N varies considerably in the literature, where emission factors have been reported from < 1-18% from under urinary N (de Klein and van Logtestijn, 1994; Carran et al., 1995; Müller, 1995; Di and Cameron, 2008); and <0.1% to 7.2% from under N fertilisers (de Klein et al., 2001; Hyde et al., 2006; Cardenas et al., 2010). In this study, the urinary and fertiliser induced N₂O emission factors (Table 3.5) ranged from 0.24-0.57% and are at the lower end of reported values. Modelled predictions of N₂O-N emission factors, using the DNDC (DeNitrification DeComposition) model, suggest in dairy farming systems, emission factors should increase with increasing fertiliser application rates (Giltrap et al., 2013). The results of this study support this only when a urine patch is not involved, and only to a limited extent because only two fertiliser rates (200 and 400 kg N ha⁻¹ yr⁻¹) were compared.

The 200N and 400N fertiliser rates did not result in cumulative N₂O emissions greater than control (background) levels, most likely because of the split application regime which saw only 25 and 50 kg N ha⁻¹ fertiliser, respectively, applied at any one time,
supporting the practice of split fertiliser applications as a practical and effective N$_2$O loss mitigation strategy (Di and Cameron, 2002b). However, greater replication may have seen differences becoming more significant.

The cumulative N$_2$O-N results (Figure 3.17) were within the range of emissions from soils treated with fertiliser and/or urine reported by other studies (von Rheinbaben, 1990). The results also support previous conclusions that (a) urine patches play a key role in N$_2$O emissions from grazed pasture systems (de Klein et al., 2001; de Klein and Ledgard, 2005; Cameron et al., 2013); and (b) N$_2$O emissions from urine patches vary considerably depending on the season in which urine deposition occurred. These results also support the theory that in general, urine deposited in the wetter, colder months of the year (autumn/winter) result in greater N$_2$O emissions than urine deposited in the drier months of the year (spring/summer) (Luo et al., 1999; de Klein et al., 2004; Di and Cameron, 2008). This is because in the spring/summer months, increased plant demand limits the amount of NO$_3^-$ substrate available for denitrifying bacteria and the drier soil conditions result in less water-filled pore space, and therefore less anaerobic sites for denitrification.

Previous field studies have reported the occurrence of peak N$_2$O emissions under urine treatments from two weeks to 2 months following urine application (de Klein et al., 2003; Van Groenigen et al., 2005; Di and Cameron, 2008). In addition, Di and Cameron (2006) reported peak N$_2$O emissions at two weeks following autumn urine application, and at two months following spring urine application, very similar to the timings observed in the current study (Figure 3.15).

The size of the N$_2$O emission peaks following urine application were similar to previously reported field measured values from free draining soils e.g. Di et al. (2007) measured a N$_2$O peak of ca. 300 g N$_2$O-N ha$^{-1}$ day$^{-1}$ under autumn applied urine on a Horotiu soil, which was very similar to that measured under autumn urine in this study (Figure 3.15). However, some other studies have reported much higher N$_2$O emission peaks of 500-2000 g N$_2$O-N ha$^{-1}$ day$^{-1}$ from under autumn applied urine (Di and Cameron, 2003; Di and Cameron, 2006; Clough et al., 2009) and up to 700 g N$_2$O-N ha$^{-1}$ day$^{-1}$ from under spring applied urine (Di and Cameron, 2003). Some of these studies had higher urine application rates of 1000 kg N ha$^{-1}$ and irrigation water was applied to the lysimeter studies (Di and Cameron (2003) and (2006)) which may have enhanced the N$_2$O emissions.
The recovery of fertiliser $^{15}$N as $\text{N}_2\text{O}^{15}$N was low, ranging from 0.025-0.04% (Figure 3.19). Other studies have observed higher $^{15}$N recovery as $\text{N}_2\text{O}$, for example Di and Cameron (2008) recovered 3.5% of $^{15}$N labelled urea and 53% of $^{15}$N labelled urine and urea (5 atom%) from a lysimeter trial. Anger et al. (2003) recovered 1.4-4.2% of fertiliser N applied as $\text{N}_2\text{O}$, and 0.3-0.9% of urine applied as $\text{N}_2\text{O}$. Wachendorf et al. (2008) recovered a similar percentage, albeit slightly higher than in this current study, of 0.05% of $^{15}$N enriched urine as $\text{N}_2\text{O}^{15}$N. When N is added to the soil system, it is partitioned between plant uptake and microbial uptake (including nitrifiers and denitrifiers) which are effectively in competition for the N substrate. There was a trend for the $\text{N}_2\text{O}^{15}$N recovery to increase with increasing fertiliser-N rate, and although this was not statistically significant, it is presumably due to more N being available for $\text{N}_2\text{O}$ production mechanisms as a result of reduced competition (i.e. increased availability) of N. However, it is important to bear in mind that only two fertiliser rates were compared (a high and average rate for dairy systems Waikato region), therefore, further work investigating a wider range of $^{15}$N enriched fertiliser rates is required to further validate this potential effect.

The greater cumulative $\text{N}_2\text{O}^{15}$N recovery from the autumn urine treatments was most likely a combination of decreased N demand from both plants and microbes enhancing substrate supply for denitrifiers and prolonged duration of moist soil conditions favouring denitrification. Also, lower soil and air temperatures (Figure 3.6) and reduced sunlight hours creating conditions less conducive to plant growth; and wetter and cooler soil conditions that were less conducive to microbial growth. The reduced N demand at this time resulted in more N available for nitrifiers and denitrifiers to produce $\text{N}_2\text{O}$.

Conversely, the lower cumulative $\text{N}_2\text{O}^{15}$N recovery from under spring applied urine was due to increased N demand and uptake by plants and soil microbes due to better growing conditions. Seasonal variation in $\text{N}_2\text{O}$ emissions under urine and/or urea fertiliser has been observed in other studies, which show greater $\text{N}_2\text{O}$ emissions during wet seasons when the soil water-filled pore space is high, and decreased $\text{N}_2\text{O}$ emissions under drier conditions (Van Groenigen et al., 2005; Luo et al., 2008).

Another possible explanation for the greater cumulative fertiliser $^{15}$N recovery as $\text{N}_2\text{O}^{15}$N emissions from the autumn urine treatments could be the introduction of a readily available C source from the urine, at a time where other conditions were non-limiting for denitrification (wetter soil conditions, mean daily temperatures between 7 and 16°C and
plentiful N substrate). The increase in readily available C would have increased the capacity for denitrification of the fertiliser associated NO₃-N, resulting in greater N₂O emissions. In addition to this there were two previous ¹⁵N enriched fertiliser applications (at the end of Feb and Mar), where the ¹⁵N may not have been completely utilised by plants and microbes, and therefore may have contributed further to ¹⁵N recovery as N₂O-¹⁵N after autumn urine application. Conversely, at the time of spring urine application, although there was an introduction of a readily available C source and N substrate in the form of urine, the soil conditions in spring were not as favourable for denitrification. There was very little rainfall in the month prior to spring urine application, and at the end of August, soil temperatures were still at winter lows (Figure 3.6). The same case is true under the nil urine treatments, where there was no C added to the system in the form of urine, and the increase in N substrate was only that which was applied as fertiliser.

3.4.3 Pasture N

Pasture N uptake is the most advantageous sink for N from both an economic and environmental perspective. Nitrogen that is utilised by the pasture in a dairy system will contribute to increased production, and limit the amount of N available to be leached and denitrified to N₂O. However, taking a whole farm system approach, the additional N in increased pasture production in a dairy system will ultimately be consumed by cattle, and since 60-90% of the N ingested by cattle is excreted in urine and dung (Haynes and Williams, 1993; Jarvis et al., 1995; Di and Cameron, 2002b), the N cycled back into the soil system will again be at risk of being leached or denitrified.

Nitrogen uptake (Figure 3.23a) followed a similar pattern to DM yield (Figure 3.21a) both temporally and cumulatively. The N content of the pasture increased considerably after the first fertiliser application and after the autumn and spring urine applications, but otherwise hovered between 2 and 4% (Figure 3.22); thus indicating that plant growth rates were a greater determinant of overall N uptake than the N content of the pasture. It is important to note the effect of pasture cover on the lysimeters in this experiment, and its potential effect on N uptake. The surface area of each lysimeter was only 0.18 m², potentially creating uncertainty when extrapolating results up to the paddock or hectare scale. For example, a single weed growing in a lysimeter can take up a significant amount of its surface area, outcompeting grass and clover, which, in turn, may affect the DM yields, N content and N uptake. Furthermore some weeds may be deeper rooting, or
have smaller surface areas than the grass and clover species, resulting in larger or smaller evaporative water loss, and potentially affecting drainage and leaching data. In this study, the weeds were sprayed to ensure a predominantly ryegrass/clover sward. The dissection data showed that grass species made up over 90% of the surface area in all lysimeters but three, F0U0 (86%), F0UA (84%) and F2UA (86%), with the remainder being made up of weeds and clover (clover made up the smallest fraction). As only one dissection was carried out during the experiment, it was not intended as a measure of how pasture species distribution changed over time, more an insight into the actual species distribution vs the lysimeter observations at a certain point in time.

The optimal mean daily temperature for ryegrass growth (the dominant species in the lysimeters) is 15-20°C (Weihing, 1963) thus the period where grass growth should have been at a maximum and where N uptake should have been greatest was from 21 Feb to 1 Apr 2011 and 1 Nov 2011–1 Apr 2012, as judged from the soil/air temperatures, provided soil moisture was not limiting (Figure 3.6).

There was a short-lived increase in pasture N uptake following autumn urine application, after which, N uptake decreased until the beginning of Sep 2011. The N content of the pasture in the autumn urine treatments increased (Figure 3.22) suggesting that luxury N uptake took place at this time. When the supply of N exceeds the short term plant requirement, the excess N taken up is stored in the plant as NO₃⁻ or amides (Whitehead, 1995) and is termed “luxury uptake”. In autumn/winter, the metabolic response of plants to N and its utilisation is lower due to lower temperatures and reduced daylight hours, and as such, the concentration of N in the plant increases. In spring 2011, pasture uptake in the autumn urine treatments increased as increasing temperatures became less limiting. Additionally, the rewetting of the soil after a dry period in August/September 2011 would have resulted in a flush of mineralisation, as well as the re-mineralisation of previously immobilised urinary N, in turn, increasing pasture N uptake. The subsequent decline in pasture N uptake after October 2011 was primarily a function of limited soil inorganic N, lower rainfall and increased evapotranspiration in late October and November (Figure 3.6).

Pasture N uptake following spring urine application was not limited by inorganic N availability, therefore it was driven by environmental conditions. Increasing temperatures and the September/October 2011 rainfall suggest that pasture N uptake over this time was
mostly uninhibited (Figure 3.6). Drier conditions in November and early December 2011 decreased pasture N uptake and the summer drainage in Dec 2011-Jan 2012 (Figure 3.6) leached the remaining NO₃⁻-N stored in the soil, further decreasing pasture N uptake (Figure 3.23a).

Following the autumn and spring urine applications, any fertiliser effects on pasture N uptake were swamped by the N loading of the applied urine. From March 2012 onwards, the increasing fertiliser effects surpassed the urine effects suggesting the majority of urinary N had either already been taken up by plants, leached, or was immobilised in the soil.

Similar to the leached NO₃⁻¹⁵N and emitted N₂O-¹⁵N, temporal ¹⁵N recovery in the pasture decreased over time, for the same reasons described earlier, because each ensuing split application of ¹⁵N enriched fertiliser increased the total mass of ¹⁵N that had been applied (increasing the denominator of Equation 3.2), and, although the ¹⁵N enrichment in each pasture harvest did not decrease over time (Figure 3.25a), the total recovery as a percentage of the ¹⁵N applied did (Figure 3.25b).

Many other studies investigating the fate of applied ¹⁵N have also found the pasture-N pool is where most ¹⁵N is recovered (Dowdell and Webster, 1980; Fraser et al., 1994; Williams and Haynes, 1994; Clough et al., 1996; Clough et al., 1998b; Decau et al., 2003; Leterme et al., 2003; Silva et al., 2005) with recoveries of 11-65%. It is important to note that all these studies (with the exception of Dowdell and Webster (1980)) measured ¹⁵N recovery in the pasture following urine-¹⁵N applications, while in this current study, the fertiliser was enriched in ¹⁵N. This, along with the fact that pasture ¹⁵N recovery increased in the harvests immediately following autumn and spring urine applications, suggests that although the soil was inundated with urine derived N, the pasture was taking up a large proportion of the fertiliser N. The proportion of unlabelled urinary N available to the pasture far exceeded the proportion of ¹⁵N enriched fertiliser N available (800 kg urine N vs 25 or 50 kg fertiliser N).

The increases in temporal fertiliser ¹⁵N recovery in the pasture in the autumn urine and spring urine treatments immediately after the respective urine application (Figure 3.25) could be due to the ponding caused during urine application resulting in preferential flow of urine deeper into the soil where it was not as readily available for plant utilisation. The fertiliser on the other hand, was washed in with 10 mm of simulated rainfall, and would
have probably been in the plant root zone during hydrolysis, and easily available for plant utilisation, hence the high fertiliser $^{15}$N recovery.

The greater $^{15}$N recovery in the spring urine treatments compared to the autumn urine treatments was a product of the greater plant growth in the spring urine treatments (Figure 3.23b). In addition, the applied urine may have caused a priming effect where the increased soil alkalinity resulting from the urine application increased the solubility of soil organic matter, enhancing soil microbial activity and thus increasing mineralisation of organic and microbial N, (including previously immobilised fertiliser $^{15}$N).

Fertiliser $^{15}$N recovered in roots and stubble made up a very small proportion of the total $^{15}$N recovery, yet collectively, it was still greater than the cumulative recovery of fertiliser as N$_2$O-$^{15}$N. Fertiliser $^{15}$N recovery in the roots and stubble was greater under the 200N rate than the 400N rate. This contradicts the above-mentioned theory that root proliferation as a result of the applied fertiliser increased the capacity of the fertiliser treatments to take up N. Since $^{15}$N recovery in the roots and stubble was measured at the end of the experiment (upon destructively coring the lysimeters), it is more indicative of recent events. The roots were sampled at the end of the 2012 winter drainage period, so perhaps some leaching of surplus fertiliser N below the plant root zone occurred in the 400N rate, while fertiliser applied at the 200N rate was efficiently used by the pasture. These results may not necessarily be indicative of the trends that were occurring throughout the experiment, or indeed relative to the cumulative pasture N uptake results previously discussed.

3.4.4 Soil N

Fertiliser $^{15}$N recovery in the soil was greatest in the top 5 cm of soil (Table 3.7). This was expected because both inorganic soil N and total (predominantly organic) soil N were also greatest in the top 5 cm of soil (Figure 3.34 and Figure 3.35). The comparatively high recovery of fertiliser $^{15}$N in the two surface layers (0-5 cm and 5-10 cm) corroborates earlier suggestions that only small amounts of fertiliser associated N were transported beyond the plant root zone, and this is supported by the high pasture $^{15}$N recovery, and comparatively low $^{15}$N recovery in the leachate and as N$_2$O emissions observed in this study. Although there was no significant treatment effect, there was a trend for greater soil fertiliser $^{15}$N recovery from the nil urine treatments compared to the autumn urine and spring urine treatments (Figure 3.38). This could be due to increased...
mineralisation of previously immobilised fertiliser-\textsuperscript{15}N due to solubilisation of soil organic matter in the urine affected treatments (unfortunately, the fate of each individual fertiliser application cannot be determined), and subsequent uptake by plants, denitrification or leaching. It could also be due to stimulated plant growth as a result of the autumn and spring applied urine, thereby resulting in greater uptake of fertiliser N, and less soil associated fertiliser N.

The soil inorganic N at the end of the experiment was dominated by the NH\textsubscript{4}\textsuperscript{+}-N fraction (Figure 3.34), presumably because the destructive coring of the lysimeters was carried out after the 2012 winter drainage period, thus the majority of the mobile soil NO\textsubscript{3}\textsuperscript{-}-N would have been flushed out, leaving NH\textsubscript{4}\textsuperscript{+}-N bound to soil exchange sites. In the top 5 cm, soil NH\textsubscript{4}\textsuperscript{+}-N was significantly greater in the treatments that did not receive urine (but did receive fertiliser) than all the autumn urine and spring urine treatments (Figure 3.32).

Bearing in mind that this result is essentially a “snapshot” of the soil N status at one time and there are no other soil results over the life of the experiment with which to compare against; this could be due to the plant root proliferation and adaptation phenomenon described above in Section 3.4.1 (Drew, 1975; Hodge, 2004). Microbial biomass N, not surprisingly, decreased with depth (Figure 3.37). It is also not surprising that no treatment effects were observed. The last split fertiliser application was applied on 31 May 2012, approximately 3 months prior to the soil analysis; and the autumn and spring urine applications occurred 16 and 12 months, respectively, prior to the soil analysis. Because no treatment effects were observed on the soil microbial biomass, it is likely that any treatment induced changes to the microbial biomass would have recovered by the time the soil cores were taken. A review of plot scale field studies by Treseder (2008) suggests that under long term N fertilisation, microbial biomass declines by an average of 15%. This was not observed in this study, in fact, as mentioned above, there were no differences in soil microbial biomass N between the soils that received urine and/or fertiliser and the control after the 19 month trial. By contrast, another study by Inselsbacher \textit{et al.} (2010) suggests that rather than declining, the soil microbial community could adapt to higher inorganic fertiliser N inputs. Inselsbacher \textit{et al.} (2010) observed that microbes were the strongest competitors for inorganic N in the first 4 hours after fertiliser application. After this, plants out-competed the microbes for the fertiliser N. Within one week 45-80\% of applied \textsuperscript{15}N was recovered in the plants, and only 1-10\% recovered in the microbial biomass (Inselsbacher \textit{et al.}, 2010). Orwin \textit{et al.} (2010)
investigated the effect of urine on soil microbial biomass under wet and dry conditions and also found that microbial biomass was initially enhanced in the presence of urine, but declined after one week in urine affected soil under wet conditions and had not recovered after 44 days; while microbial biomass was mainly unaffected by urine treated soil under dry conditions. The urine also altered the microbial community structure. The initial decline in microbial biomass is an indication that the microbial communities are under stress or are inhibited by high osmotic pressure conditions that occur as a result of hydrolysis reactions under urine/fertiliser applications (Darrah et al., 1987; Petersen et al., 2004). Changes in microbial biomass such as that observed by Orwin et al. (2010) are probably due to the build-up of N compounds e.g. NH₃, occurring as a result of increased activity of N cycling micro-organisms. Some other studies have indicated that the microbial biomass pool is not affected, or quickly recovers from the application of urine (Williams and Haynes, 2000; Nunan et al., 2006).

Although the fertiliser $^{15}$N recovery was not measured in the microbial biomass, it should be considered for future studies. The dynamics of soil microbial response to urine and fertiliser applications over time requires further research, to improve our understanding of the fate of N in pastoral systems.

### 3.4.5 Mass Balance

#### 3.4.5.1 Fertiliser $^{15}$N recovery

The total fertiliser $^{15}$N recovery ranged between 81-68%, leaving 32-19% of the fertiliser $^{15}$N unaccounted for (Table 3.8). These values were within the range of $^{15}$N recoveries reported in other studies where the fate of $^{15}$N labelled urine was determined, e.g. Decau et al. (2003) recovered 77-95%; Clough et al. (1996) recovered 44-83%; Clough et al. (1998b) recovered 68-81%; and Fraser et al. (1994) recovered 66-78%. They were also similar to $^{15}$N recoveries reported from studies of the fate of $^{15}$N labelled fertiliser e.g. Ledgard et al. (1988) recovered 62 and 76% of fertiliser $^{15}$N applied in May and June (late autumn/winter) and Dowdell and Webster (1980) recovered 45-60% of fertiliser $^{15}$N from the plant and leachate fractions only. Some studies have observed higher recoveries of 86-95%, e.g. Di et al. (2002) and Silva et al. (2005); and Ledgard et al. (1988) recovered 99% of $^{15}$N from spring applied fertiliser. Generally, the results of most of these studies are in agreement with the results of a review conducted by Allison (1955), who found that 15-20% of N in $^{15}$N balance studies was not accounted for.
Fertiliser $^{15}$N recovery was greatest from the pasture fraction (50-62%) and soil fraction (16-22%). It was much smaller in the leached inorganic N fraction (0.03-2.2%) and negligible from the N$_2$O, roots and stubble fractions (< 0.07%). This suggests that even when urine and fertiliser are applied in combination, the fertiliser N is predominantly utilised by the pasture and immobilised in the soil, while the proportions that are lost via leaching and N$_2$O emissions are minute in comparison. Furthermore, although the results show that NO$_3$-N leaching and N$_2$O emissions are significantly greater under autumn applied urine, the $^{15}$N recovery data demonstrate that this is dominated by urine associated N, and that the applied fertiliser in combination with the urine contributes very little to these pools.

With regards to the ‘unaccounted for’ $^{15}$N it should be noted that the N$_2$O monitoring in this study ceased after one year, yet the other fractions were continually measured for a further 6 months. This no doubt accounts for some of the ‘unaccounted for’ $^{15}$N fraction, but not all of it. Immediately after urine and fertiliser treatment applications, 10 mm of water was applied to supress NH$_3$ volatilisation (Black et al., 1987). Although NH$_3$ suppression was not confirmed by measurement using the enclosure technique (Black et al., 1985), other studies, e.g. Fraser et al. (1994) and Clough et al. (1996), have done so and reported minimal NH$_3$-N losses in the days following treatment application; therefore it is considered unlikely that volatilisation of $^{15}$N enriched NH$_3$ would account for any of the “missing” $^{15}$N fraction observed here.

It is most likely that the unaccounted for $^{15}$N can be attributed to gaseous N$_2$ emissions, which were not measured in this study. Very few attempts have been made to directly measure N$_2$ emissions from the field, presumably because it is notoriously difficult to do so due to the high background N$_2$ content in the atmosphere. To overcome this problem, highly enriched $^{15}$N labelling, a large soil surface area to headspace volume ratio and long measurement duration is required to achieve a sufficient differential from ambient conditions for detection (Stevens and Laughlin, 2001). The relative contribution of N$_2$ evolution to the total $^{15}$N balance requires further research. A laboratory study by Monaghan and Barraclough (1993) suggested that N$_2$ emissions could account for as much as 65% of the fate of urinary N. This work estimated that losses of N$_2$ from a urine affected soil over a 30 day period were 30-65% of the urine applied, while the contribution of N$_2$O emissions was only 1-5% of the urine applied. Work by Clough et al. (1996) also postulated that N$_2$ emissions could account for some of the missing $^{15}$N
balance. This suggestion was investigated further in a laboratory study by Clough et al. (2001), who recovered 13.3% of the $^{15}$N applied as N$_2$. There is also the possibility that gases other than N$_2$O or N$_2$ could be emitted in small amounts. Chalk and Smith, (1983) showed that nitric oxide (NO) can be produced under highly organic acid soils but generally such losses are low and in the same order of magnitude as N$_2$O.

Another possible reason for unaccounted for $^{15}$N is the entrapment of gas (predominantly N$_2$O and N$_2$) in the soil. Entrapped gas would not have been measured using the headspace chamber method. The term “entrapment” refers to N$_2$O and/or N$_2$ that is evolved deeper in the soil that must first diffuse to the soil surface before being emitted to the atmosphere. The diffusion of gases in soil, and displacement by water movement, and therefore, the magnitude of losses by these pathways are dependent on the heterogeneous distribution of air-filled pore spaces, which is itself dependent on water movement and water-filled pore spaces, which vary considerably with space and time. In addition, N$_2$O may be dissolved in water-filled pore spaces, further delaying its diffusion to the soil surface, or alternatively it may be transformed and not emitted at all. This entrapped gas may diffuse from the soil surface after gas sampling has occurred, or it may be released upon destructive soil core sampling, either way, avoiding measurement. Gas entrapment and displacement has been well documented under saturated soils and rice paddies (Lindau et al., 1988; Samson et al., 1990), and has been postulated as a source of unaccounted $^{15}$N recovery in other $^{15}$N balance studies (Clough et al., 1996), but very few attempts to measure it have been made. The key studies that have are Clough et al. (2000) and Clough et al. (2001), who in endeavouring to solve the “$^{15}$N balance enigma” found that entrapment and displacement mechanisms accounted for 9.5-23% of the $^{15}$N applied, thus potentially comprising a significant proportion of the ‘unaccounted for $^{15}$N’ fraction. Entrapment is not considered to be a likely fate in long term experiments following urine application, where there is little inorganic $^{15}$N available for denitrification, and drainage has leached and/or displaced the entrapped gasses. However, in the case of this study, the fertiliser was enriched in $^{15}$N which was continually split applied throughout the duration of the experiment, so entrapment of $^{15}$N from the later $^{15}$N fertiliser applications is a viable possibility.

The possibility of dissolved N$_2$O-$^{15}$N lost in the leachate cannot be ruled out (Dowdell et al., 1979), particularly following the application of autumn urine, when environmental conditions were favourable for denitrification, drainage and leaching were high and there
was a readily available C source from the urine application itself. Dissolved N$_2$O concentrations previously measured from a spring-fed river draining a pastoral catchment in Canterbury, New Zealand ranged from 0.67-2.0 mg N$_2$O-N L$^{-1}$, equivalent to 201-664% saturation (Clough et al., 2006; Clough et al., 2007). The highest dissolved N$_2$O concentrations were observed at the river’s spring (Clough et al., 2006) suggesting much of the observed dissolved N$_2$O in this study entered the river system through groundwater flow from the surrounding agricultural land. In the current study, dissolved N$_2$O could have been transported through the lysimeters and collected in the leachate, or NO$_3^-$-N may have been denitrified at depth. Either way, leached dissolved N$_2$O presents a viable avenue for further research into the fate of unaccounted for N in $^{15}$N mass balance studies.

One other point to consider is the leached DON fraction that was not analysed for $^{15}$N. In the autumn urine treatments, the leached DON losses were considerable, accounting for up to 10% of the apparent total N applied (Table 3.9). It is likely that the majority of this DON loss is urine associated, however, it is possible that some of the fertiliser $^{15}$N (both prior to and at the time of urine application) was rapidly immobilised by the soil microbial community, then later released and solubilised under the highly alkaline conditions as a result of hydrolysis of the urinary N, and then leached through the soil profile by the ensuing drainage. Although this is speculative; the determination of $^{15}$N recovery from the DON fraction of the leachate pool should be considered an important avenue for further research.

Interestingly, the effect of urine on total $^{15}$N was greater than the effect of fertiliser (which was only marginally significant), where the total $^{15}$N recovery was greater in the spring urine treatments than the autumn and nil urine treatments; and total $^{15}$N recovery was slightly greater in the 400N fertiliser rate treatments, than the 200N treatments. The significance of the urine effect comes from the leached inorganic N and N$_2$O fractions of the $^{15}$N balance (i.e. in all other fractions there was no urine effect) (Table 3.8).

### 3.4.5.2 Apparent N recovery

The apparent N recovery is different from the $^{15}$N recovery balance because (a) it is not quantitative, and (b) it represents the combined effects of both the urine and fertiliser treatments on the apparent fate of N.

The total apparent mass of N recovered ranged from 218 (control) to 1260 (F4UA) kg N ha$^{-1}$, with apparent total percentage recovery ranging from 65 (F4U0) to 88% (F0UA),
leaving from 12-35% of the N unaccounted for. The $^{15}$N recovery data shows that 16-22% of fertiliser $^{15}$N was recovered in the soil, predominantly as organic N, which, if added to the apparent mass recovery, would bring it close to 100%, making this a likely sink for the apparent “unaccounted for N”. Previous studies have reported similar $^{15}$N recovery values from the soil organic pool, ranging from 10-31% (Fraser et al., 1994; Clough et al., 1996; Clough et al., 1998b; Di et al., 2002; Decau et al., 2003; Leterme et al., 2003). The apparent recovery of N in the soil pool was not calculated because the large background concentration and transient nature of soil N made apparent treatment related effects difficult to determine. Dinitrogen emissions could also comprise a considerable amount of the “unaccounted for N” as shown by Monaghan and Barraclough (1993) and Clough et al. (2001).

The greater apparent N recovery in the leached inorganic fraction compared to the $^{15}$N recovery from the same fraction infers the vast majority of N leached was urine associated. Interestingly, the apparent N recovery (inorganic N + DON) was greater from the 0N rate than the 200N and 400N rates under both autumn and spring urine suggesting urinary N loss via leaching is lowered by the presence of fertiliser. This could be a result of plant root proliferation discussed earlier in Section 3.3.2 (Hutchings and de Kroon, 1994; Casper and Jackson, 1997; Hodge, 2004). The apparent N recovery in the pasture reflected this theory to some extent but not significantly so; however, there is a relationship between the N leached and pasture uptake where increased N lost via leaching results in decreased pasture uptake (Figure 3.40).
3.5 Summary

The objectives of this study were to determine the effect of urine timing on the fate of fertiliser $^{15}\text{N}$, if there was an effect of fertiliser rate and also to observe if an interaction existed between these factors. The $^{15}\text{N}$ content of the leachate, gaseous N$_2$O emissions, pasture and soil pools were determined in order to assess the fertiliser $^{15}\text{N}$ fate. The key findings are summarised below:

**Leaching**

- Fertiliser loss of NO$_3^-$-N leached was insignificant, accounting for $\leq 2.2\%$ of the fertiliser $^{15}\text{N}$ recovery.

- Urine timing affected the magnitude of fertiliser derived NO$_3^-$-N leached, which was greater under autumn urine, than spring urine events.

- Under concurrent urine + fertiliser applications, there was no effect of the 200N fertiliser rate on total NO$_3^-$-N leaching losses under autumn or spring urine. However, total NO$_3^-$-N leaching losses were increased under the 400N fertiliser rate under both autumn and spring urine applications.
• Urine timing affected the magnitude of DON leached, with the greatest DON loss measured from under autumn urine applications compared to spring. The urine increased the solubility of soil organic material, which was leached rapidly with winter drainage following autumn urine application, and stored in the soil following spring urine application to be utilised by microbes and plants.

• The results show no interaction between the fertiliser and urine treatments on leached NO$_3^-$-N or DON.

Nitrous oxide

• Losses of fertiliser N as N$_2$O (emission factors) were lower under fertiliser only treatments compared to the urine treatments.

• Urine timing affected N$_2$O losses with greater N$_2$O emissions observed from autumn urine, than spring urine. This was due to favourable conditions for denitrification following autumn urine application, and reduced N demand from pasture and soil microbes during the autumn/winter period.

• Fertiliser associated N$_2$O-$^{15}$N emissions were very low with $^{15}$N recoveries < 0.05%, suggesting N$_2$O losses were primarily urine associated.

• There was no fertiliser rate effect on N$_2$O emissions.

• There was no interaction between the fertiliser and urine treatments on N$_2$O emissions.

Pasture N

• Pasture N uptake was affected by urine timing, with greater N uptake observed from under spring urine, than autumn urine. This was due to non-limiting N availability and environmental conditions for pasture growth following spring urine application.

• Pasture uptake accounted for the greatest proportion (up to 52%) of the fertiliser $^{15}$N recovered.

• Pasture N uptake was affected by fertiliser rate, with the greatest N uptake observed from the 400N rate, followed by the 200N then 0N rates. There was no fertiliser rate effect on fertiliser $^{15}$N recovery in the pasture.
Soil N

- Urine timing had no effect on soil inorganic N, total N or microbial biomass N at the end of the experiment. There were negligible amounts of inorganic N in the soil at the end of the experiment and it is suggested that any treatment induced effects on microbial biomass had disappeared by the time of soil sampling.

- Recovery of $^{15}$N in the total soil N fraction accounted for up to 22% of the applied fertiliser $^{15}$N. Fertiliser $^{15}$N recovery was greatest in the top 10 cm of soil, suggesting little of the fertiliser applied was transported beyond the plant root zone.

- There was no effect of fertiliser rate on soil N.

- Overall, there was no interaction between the urine and fertiliser treatments on any of the soil N variables measured.
Mass Balance

- The total recovery of fertiliser $^{15}$N ranged from 68-81%, leaving 19-32% of the fertiliser $^{15}$N unaccounted for. The fate of the majority of this unaccounted $^{15}$N could be a combination of emissions as $N_2$, and leached DON.

- Urine timing affected the total fertiliser $^{15}$N recovery, with greater $^{15}$N recovered from the spring urine treatments than from the autumn urine or nil urine treatments.

- Fertiliser rate affected the total fertiliser $^{15}$N recovery such that total $^{15}$N recovery increased with increasing fertiliser rate.

- Fertiliser $^{15}$N recovery was greatest from the pasture and soil fractions, and smallest in the leached inorganic N, $N_2O$, roots and stubble fractions, thus suggesting that when fertiliser is applied over a urine patch, the fertiliser N is primarily utilised by the pasture and immobilised in the soil, while the proportions that are lost via leaching and $N_2O$ emissions are negligible.

These results reject the first hypothesis (a) that there will be an interaction between fertiliser and urine treatments on losses of fertiliser-N via leaching and $N_2O$ emissions when fertiliser is applied over a deposited urine patch. However, the second hypothesis (b), that fertiliser-N losses via leaching and $N_2O$ emissions will be greater under autumn deposited urine than spring deposited urine, is supported by these results.

The results show that even when urine and fertiliser are applied in combination, the fertiliser N is predominantly utilised by the pasture and immobilised in the soil, while the proportions that are lost via leaching and $N_2O$ emissions are small in comparison. Furthermore, although the results show that $NO_3^-$-N leaching and $N_2O$ emissions are significantly greater under autumn applied urine, the $^{15}$N recovery data demonstrate that this is dominated by urine associated N, and that the applied fertiliser directly contributes very little to these pools. However, in saying this, there is evidence to suggest that $NO_3^-$-N leaching of urine and/or soil-associated N is enhanced by the application of fertiliser at the higher (400N) rate, but not at the lower (200N) rate.

These results may have implications for the practicality of precision fertiliser application technologies. If a split application fertiliser regime was applied after grazing using urine patch avoidance technology, the results suggest that this will prevent ≤ 2% of the fertiliser
from each urine patch from being leached, and < 0.1% of the fertiliser N from being emitted as N₂O. Furthermore, although NO₃⁻-N leaching from a urine patch increased with a fertiliser application rate of 400 kg N ha⁻¹ yr⁻¹, this fertiliser rate is higher than most New Zealand dairy systems would normally apply. A more likely fertiliser rate is ≤ 200 kg N ha⁻¹ yr⁻¹, at which, no increase in NO₃⁻-N leaching was observed in this study. This raises the question of whether urine patch avoidance in precision fertiliser technology is a worthwhile pursuit. The viability and environmental benefit of such technology needs further clarification, and as such, further research into the fate of fertiliser (and urine) associated N from combined areas of urine deposition and fertiliser application is required to validate these results.
Chapter 4
Validation of a process based simulation model, APSIM, with measured data from a lysimeter study

4.1 Introduction

Experimental field trials provide valuable insight and have resulted in a significant advancement in our understanding of N transformations, movement and fate in pastoral dairy systems. However, there are many challenges associated with the undertaking of experimental work in the field, including the impracticalities associated with testing full suites of treatments (e.g. the effect of urine applied to pasture is usually established on a seasonal basis rather than having treatments for each month of the year). This usually comes down to the cost of not only setting up the trial (e.g. setting up lysimeter trials are notoriously expensive) but the on-going costs and time required for monitoring, sample collection and subsequent analyses. Other challenges can include difficult site locations, up-scaling plot measurements to paddock, field or even catchment scale, and heterogeneity at smaller scales than can be measured.

One way of overcoming these challenges is the use of agricultural models that simulate the movement and fate of nutrients in agricultural systems. Models are available to a number of end users at a lower cost than experimental campaigns and the simulations generated can provide insight and understanding of the whole system. This is particularly useful in circumstances where there is limited scope for experimental measurements and where the study of nutrient dynamics is required at larger scales than experimental campaigns typically allow. Models can also provide output data that cannot be measured experimentally (e.g. instant rates of change) and can be used in making predictions and developing hypotheses for further scientific research.

Outputs from agricultural models are being increasingly used as decision support tools for farmers, consultants and policy makers. Thus it is imperative that there is confidence in the validity of their outputs, particularly where they are relied upon as justification for on farm management decisions, or regional policy change. Characterising model performance is therefore a key part of model development, and there are a range of methods employed to achieve it (Bennett et al., 2013).
The most appropriate method for the performance evaluation of a model is determined by a range of factors including the type of model, but most importantly, the aims and objectives of the modelling exercise (Jakeman et al., 2006; Bennett et al., 2010; Bennett et al., 2013). Bennett et al. (2013) provides a comprehensive review of the methods available for quantitatively evaluating model performance. Quantitative testing is commonly used to validate models, and involves the evaluation of model output performance against experimental or observed data that was not used in the model construction (Jakeman et al., 2006). The accuracy of a model’s predictions (e.g. the fate of N) can be defined as the degree of fit between how the model represents the system’s behaviour, and our understanding of the system’s behaviour based on evidence in the form of observational data (Bennett et al., 2013).

In this study, a simulation was generated in APSIM under identical conditions, treatments and experimental procedures as those undertaken in the lysimeter experiment detailed in Chapter 3. APSIM was chosen because of its effective modular framework. Management modules within APSIM allow the user to specify rules (e.g. pasture harvest dates and residuals) that characterise and control individual simulations (Keating et al., 2003), allowing for the development of a simulation that was very similar to the experimental conditions of the lysimeter study. The objectives of this chapter were to (a) validate the modelled output data from the simulation against the experimental data gathered in Chapter 3 and identify (if any) incongruities where the modelled and measured data did not agree and the possible reasons why; and (b) determine, based on the relative agreement between the modelled and experimental data, whether the current simulation was appropriate for extrapolative analyses of the fate of N under concurrently applied urine and fertiliser in different climatic, soil and/or experimental conditions.

4.2 Model validation methods

4.2.1 Experimental approach

The methodology carried out in the lysimeter trial is described in detail in Chapter 3, however, in brief, 36 lysimeters were collected from the No. 1 Dairy Farm at the Ruakura Research Centre, Hamilton, New Zealand (latitude 37.779 °S, longitude 175.315 °E). The lysimeter collection procedure is described by Cameron et al. (1992). The lysimeters were transported and installed at the Ruakura campus, 1 km from the collection site. Urea fertiliser was applied to all lysimeters at a rate of 25 kg N ha⁻¹ to improve the pasture condition. Prior to treatment application, the lysimeters received 800 mm of irrigation water, resulting in approximately 800 mm of drainage over a period of 10 days to “flush” through any historic
urine patch effects. The experiment began on 21 February 2011 and finished on 27 August 2012. The lysimeters received treatments of $^{15}$N enriched urea fertiliser at three rates (0, 200 and 400 kg N ha$^{-1}$) applied as eight evenly split applications between August and May with and without a single urine application at a rate of 800 kg N ha$^{-1}$ in spring or autumn. There were nine treatments in total (including a control) with four replicates. Table 4.1 summarises the nine treatments, which are referred to throughout this chapter using the abbreviations shown in the “name” column. Fertiliser was applied on the following dates: 21 Feb 2011; 28 Mar 2011; 4 May 2011; 31 May 2011; 31 Aug 2011; 29 Sep 2011; 7 Nov 2011; 5 Dec 2011; 28 Feb 2012; 30 Mar 2012; 30 Apr 2012; 31 May 2012. A total of 1.8 L of cow urine was applied to the relevant lysimeters on 4th May 2011 (autumn) and 31 Aug 2011 (spring). The urine was washed in with 10 mm of water.

<table>
<thead>
<tr>
<th>Name</th>
<th>Treatment description</th>
<th>$^{15}$N Urea Rate (kg N ha$^{-1}$yr$^{-1}$)</th>
<th>Urine (kg N ha$^{-1}$yr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F0U0</td>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. F2U0</td>
<td>$^{15}$N Urea 200</td>
<td>200 (25 kg N ha$^{-1}$x 8)</td>
<td>0</td>
</tr>
<tr>
<td>3. F4U0</td>
<td>$^{15}$N Urea 400</td>
<td>400 (50 kg N ha$^{-1}$x 8)</td>
<td>0</td>
</tr>
<tr>
<td>4. F0UA</td>
<td>Urine (Autumn)</td>
<td>0</td>
<td>800</td>
</tr>
<tr>
<td>5. F2UA</td>
<td>$^{15}$N urea 200 + urine (Autumn)</td>
<td>200 (25 kg N ha$^{-1}$x 8)</td>
<td>800</td>
</tr>
<tr>
<td>6. F4UA</td>
<td>$^{15}$N urea 400 + urine (Autumn)</td>
<td>400 (50 kg N ha$^{-1}$x 8)</td>
<td>800</td>
</tr>
<tr>
<td>7. F0US</td>
<td>Urine (Spring)</td>
<td>0</td>
<td>800</td>
</tr>
<tr>
<td>8. F2US</td>
<td>$^{15}$N urea 200 + urine (Spring)</td>
<td>200 (25 kg N ha$^{-1}$x 8)</td>
<td>800</td>
</tr>
<tr>
<td>9. F4US</td>
<td>$^{15}$N urea 400 + urine (Spring)</td>
<td>400 (50 kg N ha$^{-1}$x 8)</td>
<td>800</td>
</tr>
</tbody>
</table>

Leachate was collected after each rainfall period and analysed for ammonium (NH$_4^+$-N) and nitrite + nitrate (NO$_2^-$-N+NO$_3^-$-N, referred to henceforth as NO$_3^-$-N). Nitrous oxide fluxes were measured twice weekly for the first two weeks following a fertiliser/urine application, and once weekly thereafter. In order to calculate cumulative N$_2$O fluxes, the measured fluxes were integrated using the trapezoidal method and the cumulative fluxes linearly interpolated. Lysimeter herbage was cut to a residual of 1600 kg DM ha$^{-1}$ (about 30 mm height) as required to mimic a dairy grazing regime (every 2-3 weeks in spring/autumn and every 4 weeks in summer/winter). Herbage was analysed for DM, total N and $^{15}$N atom %.

4.2.2 Model description and settings

The Agricultural Production Systems Simulator, APSIM (Keating et al., 2003) is a process-based simulation framework. The model allows for modules that represent key components
of a farming system, to be ‘plugged in’ or ‘pulled out’ by the user. APSIM was initially designed to simulate crop systems production and address associated issues. In recent years, the development of a range of plant growth modules has seen an increase in the range of applications of APSIM, including the simulation of pastoral farming systems (Snow et al., 2009; Cichota et al., 2012; Cichota et al., 2013). The key APSIM modules used in this validation study include SWIM3, SoilN and AgPasture.

SWIM3 (Huth et al., 2012) is the latest release of the SWIM (Soil Water Infiltration and Movement) models (Ross et al., 1992; Huth et al., 1996; Verburg et al., 1996a) which simulate soil water and solute movement. SWIM3 is incorporated into the APSIM framework and its primary role is to calculate fluxes and storage of soil water and solutes and communicate this information to other modules in the simulation (Huth et al., 2012). The functions of SWIM3 are also detailed in Appendix A. The physical components and flows of the soil-water system modelled by SWIM3 are illustrated in Figure 4.1 below:

![Figure 4.1 Components of the soil water and solute balance that are addressed by SWIM (Verburg et al., 1996a).](image)

Water fluxes in soil are calculated in SWIM3 through a numerical solution to the Richards equation (Equation 4.1) (Richards, 1931):

\[
\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x} \left[ K (\psi) \left( \frac{\partial \psi}{\partial x} + \frac{\partial z}{\partial x} \right) \right] + S
\]

Where \( \theta \) = volumetric water content (\( \text{cm}^3 \text{ cm}^{-3} \)), \( x \) = space (cm), \( t \) = time (h), \( K \) = hydraulic conductivity (\( \text{cm} \text{ h}^{-1} \)), \( z \) = gravitational potential (cm), \( \psi \) = matric potential (cm), and \( S \) =
source/sink term for water (cm³ cm⁻³ h⁻¹) (Huth et al., 2012). Solute fluxes are calculated through a solution to the convection-dispersion equation (Equation 4.2).

\[
\frac{\partial (\theta c)}{\partial t} + \frac{\partial (\rho s)}{\partial t} = \frac{\partial}{\partial x} \left( \theta D \frac{\partial c}{\partial x} \right) - \frac{\partial (qc)}{\partial x} + \varphi
\]

Where \( c = \) solute concentration in solution (ppm), \( s = \) solute concentration adsorbed to the soil surface (ppm), \( D = \) the combined dispersion and diffusion coefficient (cm² h⁻¹), \( q = \) water flux (cm h⁻¹), \( \rho = \) soil bulk density (g cm⁻³), \( \varphi = \) source/sink term for solute (ppm h⁻¹) (Huth et al., 2012). Verburg et al. (1996a) provides a detailed description of the steps used in solving these equations.

SoilN (Probert et al., 1998) estimates both organic and mineral C and N dynamics in soil layers and is also described in Appendix A. The organic C and N components transfer between four conceptual pools: microbial biomass, fresh organic matter, humus, and inert organic matter. Mineral C and N transformations include urea hydrolysis, nitrification and denitrification. Urea hydrolysis and denitrification use first order processes based on pH, organic matter concentration, soil temperature and soil moisture. Nitrification is calculated using the Michaelis-Menton equation. All mineral transformations are affected by soil water, temperature and pH (Probert et al., 1998).

The AgPasture module (Li et al., 2011) is also described in Appendix A. AgPasture was adapted from the pasture model from EcoMod and DairyMod and enables pasture based systems to be modelled within APSIM, in combination with other land uses. AgPasture integrates a range of pasture species within the module which compete for resources (e.g. radiation, water, N) and their functions and processes include photosynthesis, respiration, biomass accumulation, biomass partitioning across different organs, litter deposition and root senescence (Li et al., 2011). Full documentation on AgPasture can be located at www.apsim.info/Wiki/AgPasture.ashx.

A series of nine base simulations (one simulation for each treatment) were set up in APSIM using default parameter values with the exception of the soil properties described above and climate data. A ryegrass/white clover sward was used, assuming 100% pasture cover and no weeds at initialisation. Each simulation ran from 15 December 2010 (the date the lysimeters were installed) to 27 August 2012. The lysimeter installation date was used rather than the experiment start date to incorporate the pre-experimental conditions into the simulation, particularly the pre-experimental drainage. The correct rate of urea fertiliser was applied to the appropriate simulations on the dates specified above by inserting 20% of the fertiliser at 5
mm soil depth, 40% at 10 mm and the remaining 40% at 20 mm depth. Simulated urine was applied to the appropriate simulations on the dates specified above by inserting 1.8 L of water and the equivalent of 800 kg N ha\(^{-1}\) urea to the soil. Upon application, the simulated urine N concentration reached an initial depth of 150 mm. Simulated pasture was harvested to a residual biomass of 1600 kg DM ha\(^{-1}\) on the same dates as in the experiment. The pre-experimental water application and the 10 mm water added to the lysimeters after each fertiliser/urine application were simulated as irrigation events, applying the appropriate amount of water (mm) on the appropriate date.

The following outputs were analysed from each simulation and compared to the experimental data: drainage, leached NO\(_3^–\)N, leached NH\(_4^+\)-N, dry matter (DM) yield, N uptake, and N\(_2\)O-N emissions. These results are presented over time as both daily and cumulative data. The following outputs from the simulation were also analysed, where experimental measurements were not obtained: N\(_2\) emissions, total denitrification, N\(_2\)O emissions from denitrification and nitrification, depth of N\(_2\)O production, NH\(_3\) volatilisation, soil active C, soil urea, soil NO\(_3^–\)-N, soil NH\(_4^+\)-N and volumetric soil water content.

### 4.2.3 Soils and climate

Soil for the lysimeter experiment was collected from Ruakura, Hamilton, New Zealand. Ruakura is located in the Hamilton basin, which is an extensive depression filled with alluvium deposited by the Waikato River. A single soil type, the Horotiu silt loam (Typic orthic allophonic soil) (Hewitt, 1998) was used. The Horotiu silt loam characteristics have been described previously in Section 3.2.1. The observed soil profile at the lysimeter collection site (Figure 4.2b), was reasonably well matched to the description given by Singleton (1991) (Figure 4.2a).
Figure 4.2  (a) Horotiu silt loam profile (Waikato Regional Council, 2011) and (b) soil horizon profile at lysimeter collection site. Both profiles are the same scale.

The Horotiu soil properties used in the simulation were compiled by R. Cichota and V. Snow (AgResearch, pers.comm. 2011) and the basic soil properties are outlined in Table 4.2. The soil hydraulic properties were acquired from Vogeler (2007) and the remaining soil properties were obtained from Close et al. (2003). Values for total C were calculated as a function of depth as described by (Cichota et al., 2013). Total C was separated into the three fractions required by the SoilN module (Probert et al., 1998). The biomass C fraction was assumed to be 6.8% of the active C at the soil surface, decreasing exponentially with depth. The inert C fraction was assumed to be the average of two values: (a) a function of soil depth from a value of 25% at the soil surface, decreasing exponentially to 99% below the root zone (700 mm), and (b) the assumption that 10% of the total C was active. The active humus fraction was calculated as the difference between the other two fractions.

Table 4.2 Basic soil properties by horizon, used in the simulation (Close et al., 2003).

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>Texture (%)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Bulk density (g cm⁻³)</th>
<th>Organic Carbon (%)</th>
<th>Total Nitrogen (%)</th>
<th>CEC (meq/100g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-60</td>
<td></td>
<td>33.77</td>
<td>48.62</td>
<td>17.61</td>
<td>0.865</td>
<td>8.2</td>
<td>0.77</td>
<td>28.2</td>
<td>5.7</td>
</tr>
<tr>
<td>60-170</td>
<td></td>
<td>33.91</td>
<td>49.73</td>
<td>16.36</td>
<td>0.835</td>
<td>5.5</td>
<td>0.55</td>
<td>21.0</td>
<td>5.3</td>
</tr>
<tr>
<td>170-310</td>
<td></td>
<td>35.91</td>
<td>47.67</td>
<td>16.42</td>
<td>0.805</td>
<td>3.3</td>
<td>0.32</td>
<td>17.3</td>
<td>5.9</td>
</tr>
<tr>
<td>310-550</td>
<td></td>
<td>46.26</td>
<td>24.71</td>
<td>29.03</td>
<td>0.830</td>
<td>1.7</td>
<td>0.15</td>
<td>12.0</td>
<td>6.6</td>
</tr>
<tr>
<td>550-700</td>
<td></td>
<td>53.54</td>
<td>28.02</td>
<td>18.44</td>
<td>0.820</td>
<td>1.7</td>
<td>0.14</td>
<td>12.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 4.3 Soil hydraulic properties by horizon, used in the simulation (Vogeler, 2007).

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>Soil water content (cm³ cm⁻³)</th>
<th>Ks (mm h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAT(a)</td>
<td>DUL(b)</td>
</tr>
<tr>
<td>0-60</td>
<td>0.58</td>
<td>0.565</td>
</tr>
<tr>
<td>60-170</td>
<td>0.606</td>
<td>0.515</td>
</tr>
<tr>
<td>170-310</td>
<td>0.617</td>
<td>0.514</td>
</tr>
<tr>
<td>310-550</td>
<td>0.62</td>
<td>0.475</td>
</tr>
<tr>
<td>550-700</td>
<td>0.625</td>
<td>0.481</td>
</tr>
</tbody>
</table>

(a) Saturation; (b) Drained upper limit (field capacity); (c) Lower limit for plant uptake at 15 bar; (d) air dried soil; (e) saturated hydraulic conductivity.

The soil nutrient dynamics and hydraulic properties in APSIM were simulated at 14 detailed depth intervals over the 700 mm profile. This level of detail was required for accurate
simulation of soil processes, but was not practical for reporting, therefore, the outputs were, in most cases, reported at four depth intervals of 0-20, 20-100, 100-200 and 200-700 mm.

Rainfall at Ruakura is seasonally distributed. The wettest months are typically June and July and rainfall generally exceeds evapotranspiration during these months resulting in soil drainage. The driest months are usually February and March where evapotranspiration exceeds rainfall resulting in a soil water deficit. Average annual rainfall is 1,200 mm (Singleton, 1991). Total rainfall was 2,230 mm over the entire 19 month experiment, with 1410 mm rainfall in year one, and 820 mm in the remaining seven months. There was uncharacteristically high rainfall in December 2011 (220 mm) and early January 2012 (130 mm) with both events resulting in summer drainage (Figure 4.3).

Ruakura has a mild temperate climate with an average daily temperature of 13.9°C. The warmest months are January and February (average temperature of 18°C) and the coldest months are June, July and August (average temperature of 8-9 °C) (Singleton, 1991). Temperature in the topsoil is slightly higher than air temperature, due to the soil’s ability to retain solar heat, but follows similar diurnal fluctuations. Daily temperature fluctuations in the upper subsoil are significantly less due to lag of heat conduction, and subsoil below 0.5 m only follows monthly and seasonal air temperature changes (Singleton, 1991). Frosts occur frequently at Ruakura with the earliest ranging from March to August, and the latest ranging from June to November. The average frost free period at Ruakura is 228 consecutive days (Singleton, 1991). Daily weather data for Ruakura (station #26117) was obtained from the National Climate Database (CliFlo) (NIWA, 2007), and the rainfall, air temperature, soil temperature (100 mm depth) and evapotranspiration are shown in Figure 4.3.
4.2.4 Statistical analysis

Where the daily modelled outputs from APSIM were compared with experimental data from the lysimeter trial, the root mean square error (RMSE) was calculated for each variable of each treatment (Equation 4.3). The RMSE expressed the mean error of the model-predicted vs mean experimental values in the same units as the output of interest. Calculation of the RMSE firstly involved the summation of the individual squared errors (total squared error) for each replicated variable in each treatment. Squaring the residuals before calculating the mean generated positive values with the larger errors having a greater influence on the total square error (Bennett et al., 2013). The total square error was divided by \( n \), giving the mean square error (MSE), and finally the square root of the MSE was taken, giving the RMSE (Willmott and Matsuura, 2005). The mean of the four RMSE values for each replicate was taken, to give a single RMSE value for each treatment of each output variable, and this is presented on the temporal graphs.

\[
RMSE = \sqrt{\frac{\sum_{t=1}^{n} (y_t - \hat{y}_t)^2}{n}}
\]
Where $y_t$ = the experimental variable (dependent variable) and $\hat{y}_t$ = the simulated value for time $t$; and $n$ = the number of predictions. A lower RMSE indicated a smaller difference between the modelled and experimental values (i.e. a better ‘fit’).

For the comparison of the cumulative modelled and experimental data, an RMSE was not appropriate because the nature of cumulative data is that it cannot fluctuate (i.e. it can only increase with time) therefore, the increases and decreases that occur in variables over time are not reflected to the same extent. However, an RMSE was calculated for the final cumulative values as an indication of the difference between the cumulative modelled and experimental values. In addition, the upper and lower 95% confidence intervals for the mean of the experimental data were calculated, and are presented in the cumulative graphs. Generally, if the cumulative simulated values are within the 95% confidence interval of the mean experimental values, they can be regarded as a ‘good fit’.
4.3 Results

Comparisons of simulated and experimental variables were made on both a temporal and cumulative basis. The RMSE was calculated for the daily comparisons, and is displayed on all relevant figures, but, as mentioned above, was not appropriate for determining error in the cumulative data. In comparing the cumulative data, the raw experimental values (4 replicate values) for each variable were compared to the simulated value, with the figures giving a visual indication of where the simulated value was in relation to the experimental values.

4.3.1 Pasture growth and N uptake

Temporal

Pasture yield and N uptake followed very similar patterns over time in both the modelled and measured data. In all treatments, the model underestimated pasture yield and N uptake over the winter months (from 29 April to 28 July 2011). There was a peak in pasture yield and N uptake on 31 May 2011 that the model considerably underestimated in most treatments (Figure 4.4 and Figure 4.6).

In the spring urine treatments, the model continued to underestimate pasture yield and N uptake until 3 Nov 2011, after which, it was overestimated until 1 April 2012. In all the other treatments, from 1 Sep 2011 onwards, the model tended to overestimate pasture yield and N uptake until 1 April 2012. The exception was two peaks, on 16 Oct 2011 and 19 Dec 2011, in the F2U0, F4U0, F2UA and F4UA treatments, where the model underestimated pasture yield and N uptake on these harvests (Figure 4.4 and Figure 4.6). The model underestimated pasture yield and N uptake in all treatments (except F0U0) from 1 April 2012 onwards (Figure 4.4 and Figure 4.6).

Cumulative

Cumulative pasture yield and N uptake were very similar in both the modelled and measured data. The modelled cumulative DM yields were all within or very close to the 95% confidence interval of the mean of the measured data in all treatments except F2U0 and F4U0, which were underestimated by the model (Figure 4.5 and Figure 4.7). The models under-prediction of yield and N uptake in the winter months from around 1 May to 30 Sep 2011 was also evident in the cumulative data, with the over-prediction in the spring/summer months from 1 Oct 2011 to 1 Jan 2012 ‘catching up’ to the measured cumulative data by the end of the experiment (Figure 4.5 and Figure 4.7).
Figure 4.4 Daily DM yield simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 20.
Figure 4.5 Cumulative pasture DM yield simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only.
Figure 4.6  Daily pasture N uptake simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 19.
Figure 4.7 Cumulative pasture N uptake simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only.
4.3.2 Water and solute movement through soil

4.3.2.1 Drainage

Temporal

At the first measured drainage event on 28 Mar 2011, the model did not produce drainage in any of the treatments (Figure 4.8). At the following drainage event on 29 Apr 2011, the model underestimated drainage by 20-40 mm in all treatments (Figure 4.8). After this, the modelled drainage was close to the measured drainage until 8 Aug 2011 (within 10 mm), after which, drainage tended to be underestimated in all treatments, until about 20 June 2012 (Figure 4.8). After this, simulated drainage was very close to measured drainage, with the exception being an overestimate of the drainage event on 23 July 2012 and an underestimate of the drainage event on 3 Aug 2012 (Figure 4.8).

Cumulative

The cumulative modelled drainage was within the 95% confidence interval of the mean of the measured data in all treatments except the autumn urine treatments, F0UA, F2UA and F4UA (Figure 4.9). Cumulative drainage in these treatments was slightly underestimated by the model for the duration of the experiment (Figure 4.9).
Figure 4.8 Daily drainage simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 21.
Figure 4.9 Cumulative drainage simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only.
4.3.2.2 Leached NH$_4^+$-N

Temporal and Cumulative

The model calculated zero NH$_4^+$-N leaching throughout the course of the experiment. There were some peaks of leached NH$_4^+$-N measured from some of the treatments; however, these occurred in only 1 of the 4 lysimeter replicates, with the majority being < 4 kg ha$^{-1}$. Moreover, there was no treatment effect at any time on measured leached NH$_4^+$-N. For this reason, the leached NH$_4^+$-N data is not included or discussed any further here.

4.3.2.3 Leached NO$_3^-$-N

Temporal

The model calculated zero NO$_3^-$-N leaching over cumulative drainage from the treatments that received no urine. This was in fact the case for the control (F0U0), but NO$_3^-$-N leaching ranged from 0-0.5 kg ha$^{-1}$ and 0-6 kg ha$^{-1}$ in the F2U0 and F4U0 treatments, respectively (Figure 4.10). In the autumn urine treatments, the model predicted NO$_3^-$-N leaching to start after around 70 mm drainage, but measured leaching occurred after 180 mm drainage. The highest peak NO$_3^-$-N flux in the modelled data occurred at around 400 mm drainage, whereas the measured peak occurred at 460 mm drainage in all the autumn urine treatments (Figure 4.10). The modelled NO$_3^-$-N concentration peaks were underestimated by the model in the autumn urine treatments by 58, 28 and 58 kg NO$_3^-$-N ha$^{-1}$ in the F0UA, F2UA and F4UA treatments, respectively (Figure 4.10). In the spring urine treatments, simulated leached NO$_3^-$-N was observed after around 430 mm cumulative drainage. This was earlier than the measured leached NO$_3^-$-N which was observed after 500 mm drainage in the F0US and F2US treatments, and 460 mm drainage in the F4US treatment (Figure 4.10). The simulated NO$_3^-$-N peak concentrations were also overestimated by 5 and 8 kg NO$_3^-$-N ha$^{-1}$ in the F0US and F4US treatments, respectively, compared with the measured NO$_3^-$-N peaks (Figure 4.10). There was no distinctive NO$_3^-$-N peak measured in the F2US treatment, and the model overestimated the highest mean concentration by 25 kg NO$_3^-$-N ha$^{-1}$ (Figure 4.10).

Cumulative

As described in the temporal data, the model calculated zero cumulative NO$_3^-$-N loss in the treatments that did not receive urine (F0U0, F2U0, and F4U0). This was still within the 95% confidence interval of the mean of the measured data in the F0U0 and F4U0 treatments, and just outside that of the F2U0 treatment. Measured cumulative NO$_3^-$-N leached ranged from 0 to 1, 5 and 28 kg NO$_3^-$-N ha$^{-1}$ in the F0U0, F2U0 and F4U0 treatments, respectively (Figure 4.11). The value of 28 kg NO$_3^-$-N leached from F4U0 was due to an outlier from a single
replicate and was reanalysed to ensure the validity of the result. Without the outlier present, there was no more NO$_3^-$-N leached from F4U0 than F2U0.

In the autumn urine treatments, the modelled cumulative NO$_3^-$-N leached was slightly below the 95% confidence interval of the mean of the measured data in the F0UA and F2UA treatments, and within that of the F4UA treatment, though at the lower end (Figure 4.11). Nearly all the modelled leached NO$_3^-$-N occurred between 200 and 450 mm drainage (31 May and 31 Jul 2011) with little to no further loss after this. The cumulative measured NO$_3^-$-N leached was lost between 300 and 550 mm drainage (Figure 4.11). In other words, the amount of drainage over which the majority of NO$_3^-$-N was leached was the same in both the modelled and measured data, but the onset of drainage occurred earlier in the modelled drainage, which can be seen more clearly in the temporal data (Figure 4.10).

In the spring urine affected treatments, the cumulative modelled NO$_3^-$-N leached values were all within the 95% confidence interval of the mean measured data (Figure 4.11), although the scatter of the measured data increased considerably after 1 Jan 2011. The onset of modelled NO$_3^-$-N leaching started at ~ 500 mm, similar to the measured cumulative NO$_3^-$-N leaching in the F2US and F4US treatments, and slightly later than the F0US treatment. However, the modelled leaching finished at 530 mm, while in the cumulative measured data, NO$_3^-$-N leaching ceased at between 535-930 mm (Figure 4.11).
Figure 4.10 Daily leached NO₃⁻N flux simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over cumulative drainage. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 21.
Figure 4.11 Cumulative leached NO$_3$–N vs cumulative drainage simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments. Note: F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. Also note the Y axes are different.
4.3.3 Ammonia Volatilisation

Ammonia volatilisation was not measured during the lysimeter experiment; however, it was simulated by APSIM over the same time period. Cumulative NH$_3$ volatilisation from treatments that received nil urine was negligible and all values were $\leq 0.004$ kg NH$_3$-N ha$^{-1}$ (Figure 4.12). In the autumn urine treatments, cumulative NH$_3$-N volatilisation increased from 0 to 13, 14 and 16 kg NH$_3$-N ha$^{-1}$ in the F0UA, F2UA and F4UA treatments, respectively over the three days following urine application (Figure 4.12). In the spring urine treatments, NH$_3$-N volatilisation increased from 0 to 22, 23 and 26 kg NH$_3$-N ha$^{-1}$ in the F0UA, F2UA and F4UA treatments, respectively, over the three days following urine application. These elevated NH$_3$ emissions were short-lived and all treatments returned back to zero within six days of urine application, with the exception of F4US where there were some small NH$_3$ emissions in the months following urine application (Figure 4.12).

![Figure 4.12 Cumulative NH$_3$-N volatilisation over time for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$, respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications.](image)

4.3.4 Nitrous oxide emissions

Temporal

The model calculated zero N$_2$O emissions from the F0U0 and F2U0 treatments, while N$_2$O-N emissions of between 0 and 0.04 kg ha$^{-1}$ were measured at various times throughout the
experiment. In the F4U0 treatment, the simulation modelled a single peak of 0.1 kg N₂O-N ha⁻¹ emissions on 6 June 2011. This was about 4 times the size of the largest measured N₂O fluxes, with no other modelled N₂O emissions for the remainder of the experiment (Figure 4.13). In the autumn urine treatments, the model calculated large N₂O peaks, starting on 4 May 2011 (the day of autumn urine application), peaking at 0.5 kg N₂O-N ha⁻¹ in all three treatments on 31 May 2011, and returning to 0 on 24 Oct 2011, with no further emissions after this (Figure 4.13). However, the measured N₂O fluxes associated with urine were much smaller, and reached 0.3, 0.2 and 0.1 kg N₂O-N ha⁻¹ in the F0U0, F2U0 and F4U0 treatments, respectively, returning to background levels by late June, with fertiliser and non-fertiliser associated fluctuations in N₂O emissions occurring to the end of the experiment (Figure 4.13).

In the spring urine treatments, the model calculated zero N₂O-N emissions in the F0U0 and F2US treatments until 1 Sep 2011, and one small peak of 0.1 kg N₂O-N ha⁻¹ on 6 June 2011 in the F4US treatment (Figure 4.13). After spring urine application, the model calculated two N₂O peaks, with the first reaching 0.4, 0.4 and 0.5 kg N₂O-N ha⁻¹ on 7 Nov 2011 in the F0US, F2US and F4US treatments, respectively, and returning to zero by 11 Nov 2011. The second peaks reached 0.4, 0.4 and 0.5 kg N₂O-N ha⁻¹ on 18 Dec 2011 in the F0US, F2US and F4US treatments, respectively, returning to zero by 20 Jan 2012 for the remainder of the experiment (Figure 4.13). There were also two spring urine associated N₂O-N peaks in the measured data, however these were much lower and short-lived at 0.06, 0.05 and 0.03 kg N₂O-N ha⁻¹ on 8 Sep 2011, and 0.14, 0.08 and 0.05 kg N₂O-N ha⁻¹ on 2 Oct 2011 in the F0US, F2US and F4US treatments, respectively, with the emissions returning to background levels by 12 Oct 2011 (Figure 4.13). Both prior to and following the spring urine associated peak, there were lower fertiliser and non-fertiliser associated N₂O-N emissions throughout the experiment (Figure 4.13).

Cumulative

Cumulative N₂O-N emissions were underestimated by between 0.5 and 2 kg N₂O-N ha⁻¹ in the model in the treatments that received no urine (Figure 4.14). On the other hand, APSIM significantly overestimated cumulative N₂O-N emissions from all the treatments that received urine. In the case of the autumn urine treatments, the modelled cumulative N₂O-N emissions were 34, 36 and 38 kg N₂O-N ha⁻¹ greater than the measured N₂O-N emissions from the F0UA, F2UA and F4UA treatments, respectively (Figure 4.14). In the spring urine treatments the modelled cumulative N₂O-N emissions were 12, 12 and 13 kg N₂O-N ha⁻¹ greater than the measured N₂O-N emissions from the F0US, F2US and F4US treatments, respectively (Figure 4.14).
Figure 4.13 Daily N2O emissions simulated in APSIM (solid line) and measured (mean) from lysimeter experiment (dotted line) for all treatments over time. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine+fertiliser application; other arrows indicate split fertiliser applications. Error bars indicate the RMSE (Root Mean Square Error) between modelled and measured values, n = 371.
Figure 4.14 Cumulative N₂O emissions simulated in APSIM (solid lines) and measured from lysimeter experiment (diamonds) for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + fertiliser application; other arrows indicate split fertiliser applications. The error bars indicate the RMSE (Root Mean Square Error) for the final cumulative values only. Also note the Y axes are different.
4.3.5 Total denitrification

Denitrification was not measured during the lysimeter experiment, but was simulated in APSIM (Figure 4.15). The relative proportions of N₂O and dinitrogen (N₂) produced during denitrification were also simulated (Figure 4.16). There was zero total denitrification in the F0U0 treatment and very little in the other non-urine treatments with F2U0 reaching <1 and F4U0 reaching 1.7 kg cumulative N denitrified ha⁻¹. In the autumn urine treatments, denitrification increased immediately following urine application reaching a maximum in early Oct 2011 of 216, 227 and 238 kg N ha⁻¹ denitrified in the F0UA, F2UA and F4UA treatments, respectively. In the spring urine treatments, denitrification reached about 50 kg N ha⁻¹ for all treatments on 12 Nov 2011 then plateaued. A second pulse of denitrification occurred between 1 Dec 2011 and 30 Jan 2012 as a result of the high summer rainfall resulting in a total cumulative N denitrified of 86, 89 and 94 kg ha⁻¹ in the F0US, F2US and F4US treatments, respectively.

Dinitrogen emissions were the dominant gas product of modelled denitrification in all treatments. In F2U0 and F4U0, N₂ was the only gas produced, emitting 0.21 and 1.7 kg cumulative N₂ ha⁻¹, respectively. In the urine affected treatments, cumulative N₂ emissions were 175, 184 and 193 kg ha⁻¹ in the F0UA, F2UA, and F4UA treatments, respectively, and 70, 72, and 76 kg ha⁻¹ in the F0US, F2US, and F4US treatments, respectively. Table 4.4 shows a summary of values for modelled denitrification and the associated gaseous products along with the measured N₂O emissions from the lysimeter experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total denitrification (kg N ha⁻¹)</th>
<th>N₂ produced (kg N₂ ha⁻¹)</th>
<th>N₂O produced (kg N₂O-N ha⁻¹)</th>
<th>Range of N₂O produced (kg N₂O-N ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0U0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.5 - 1.1</td>
</tr>
<tr>
<td>F2U0</td>
<td>0.21</td>
<td>0.21</td>
<td>0.00</td>
<td>1.1 - 1.7</td>
</tr>
<tr>
<td>F4U0</td>
<td>1.70</td>
<td>1.70</td>
<td>0.00</td>
<td>1.5 - 2.0</td>
</tr>
<tr>
<td>F0UA</td>
<td>216</td>
<td>175</td>
<td>41.6</td>
<td>4.0 - 7.8</td>
</tr>
<tr>
<td>F2UA</td>
<td>227</td>
<td>184</td>
<td>43.4</td>
<td>4.8 - 7.9</td>
</tr>
<tr>
<td>F4UA</td>
<td>238</td>
<td>193</td>
<td>45.1</td>
<td>4.9 - 8.5</td>
</tr>
<tr>
<td>F0US</td>
<td>86.0</td>
<td>70.0</td>
<td>16.0</td>
<td>2.3 - 4.9</td>
</tr>
<tr>
<td>F2US</td>
<td>88.5</td>
<td>71.8</td>
<td>16.7</td>
<td>2.5 - 5.2</td>
</tr>
<tr>
<td>F4US</td>
<td>93.5</td>
<td>76.3</td>
<td>17.2</td>
<td>3.1 - 5.1</td>
</tr>
</tbody>
</table>
Figure 4.15 Cumulative denitrification simulated in APSIM for all treatments. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\); and U0, UA and US denote nil, autumn and spring urine applications, respectively. Bold arrows indicate urine + fertiliser application in either autumn or spring; other arrows indicate split fertiliser applications.

Figure 4.16 Total denitrification simulated in APSIM with relative proportions of N\(_2\)O and N\(_2\) products for all treatments.
4.3.6 Denitrification vs nitrification derived nitrous oxide emissions

The relative contribution of denitrification and nitrification to the N\textsubscript{2}O emissions was not determined in the lysimeter experiment. However, APSIM calculated that all emissions were due to denitrification, with zero emissions calculated from nitrification.

4.3.7 Depth of nitrous oxide production

Although N\textsubscript{2}O emissions were measured from the surface of the lysimeters, this gave no indication as to the depth at which the N\textsubscript{2}O was evolved. Nitrous oxide production was calculated in APSIM at 14 depth increments with the sum of these being the total N\textsubscript{2}O emissions. The N\textsubscript{2}O produced in the simulation at each of these 14 depths is presented in Figure 4.17. There was negligible N\textsubscript{2}O produced in the treatments that received nil urine (F0U0, F2U0 and F4U0), therefore these treatments are not displayed in Figure 4.17.

In all treatments, the greatest N\textsubscript{2}O production occurred at the 100-150 mm depth range with 1 kg N\textsubscript{2}O produced per cm soil in the autumn urine treatments, and 0.5 kg N\textsubscript{2}O ha\textsuperscript{-1} produced per cm soil in the spring urine treatments. In all treatments, N\textsubscript{2}O production considerably declined in the 300-400 mm zone, however, in the autumn urine treatments, N\textsubscript{2}O production increased with increasing depth below this down to 700 mm, with around 0.85 kg N\textsubscript{2}O ha\textsuperscript{-1} produced per cm soil at the 650-700 mm depth increment (Figure 4.17). This increase in N\textsubscript{2}O production in the deeper zones did not occur in the spring urine treatments. From 300 mm downwards, N\textsubscript{2}O production in the spring urine treatments remained at around 0.15 kg N\textsubscript{2}O ha\textsuperscript{-1} per 10 mm soil, with a small increase to 0.19 kg N\textsubscript{2}O ha\textsuperscript{-1} per cm soil, in the bottom 650-700 mm zone Figure 4.17.

Approximately 50 and 40% of the total N\textsubscript{2}O produced was evolved at between 200-700 mm depth in the autumn and spring urine treatments, respectively. The remaining 50 and 60% was produced above 200 mm depth (Figure 4.17).
Figure 4.17  Total N₂O production simulated by APSIM at incrementing depths from 0-700 mm. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹; and U0, UA and US denote nil, autumn and spring urine applications, respectively.

4.3.8 Soil active carbon

Soil ‘active C’ is defined as the C which is available as a substrate for denitrification in APSIM. Active C (Cₐ) is calculated in APSIM using Equations 4.4 and 4.5 (Thorburn et al., 2010).
\[ C_{A,i} = 0.0031 \times SOC_{ppm,i} + 24.5 \]

Where \( SOC_{ppm} \) = the soil organic C in the \( ith \) soil layer, and is calculated by:

\[ SOC_{ppm,i} = HUM_{Cppm,i} + FOM_{Cppm,i} \]

Where \( HUM_{C} \) = the humic C pool and \( FOM_{C} \) = the fresh organic matter C pool in the \( ith \) soil layer.

Soil active C was not measured during the experiment, but was calculated by APSIM for the experimental period (Thorburn et al., 2010). The amount of active C in each soil layer is a limiting factor for \( N_2O \) production, therefore, it is presented in the same detailed soil layers as the \( N_2O \) production above. Soil active C was calculated from the HUM_C and FOM_C outputs from APSIM (kg ha\(^{-1}\)) and converted to ppm based on the bulk density of each soil layer.

Soil active C did not fluctuate over time. The smallest and largest range in soil C in any given depth over the duration of the experiment was 0.1 and 1.53 mg kg\(^{-1}\) active C per cm soil, respectively. Given that the soil active C ranged from 82-531 mg kg\(^{-1}\) per cm soil (Figure 4.18), this variation was considered negligible. Simulated soil active C did not differ with treatment, and decreased from 531 ppm per cm soil in the surface 1 cm depth, to 82 mg kg\(^{-1}\) per cm at the 40-50 cm depth, and back up to 112 mg kg\(^{-1}\) per cm from the 50 cm depth below (Figure 4.18).
4.3.9 Soil inorganic N

Inorganic soil N was not measured during the experiment as it would have had a destructive effect on the soil profile within the lysimeters, and would have jeopardised the accuracy of gas measurements and pasture yields. However, the modelled soil N data provides information on how APSIM partitions N that is applied to the soil. Soil urea (Figure 4.19), soil NH$_4^+$-N (Figure 4.20), and soil NO$_3^-$-N (Figure 4.21) were modelled by APSIM in multiple soil layers over time and are reported in four depth intervals: 0-20, 20-100, 100-200 and 200-700 mm.

Soil urea was very short-lived reaching between 3-13 kg urea-N ha$^{-1}$ in the fertiliser only treatments; between 325-335 kg urea-N ha$^{-1}$ in the autumn urine treatments and between 400-409 kg urea-N ha$^{-1}$ in the spring urine treatments, immediately after urine application. In the nil urine and autumn urine treatments, soil urea returned to a background level of zero between 2 and 5 days. In the spring urine treatments, hydrolysis took longer, with soil urea-N returning to background levels after 9 days.

With regards to soil NH$_4^+$-N, all urine-affected treatments reached a total peak of between 600 and 670 kg NH$_4^+$-N ha$^{-1}$. Soil NH$_4^+$-N increased immediately following urine application, and peaked (indicating complete hydrolysis) within 3 days, with the elevated concentrations
lasting up to 4 months in both the autumn and spring urine treatments (Figure 4.20). In all treatments, except F0U0, there were smaller peaks of up to 5 and 14 kg NH$_4^+$-N that coincided with the fertiliser application rates of 200 and 400 kg N ha$^{-1}$, respectively. The model calculated zero kg NH$_4^+$-N ha$^{-1}$ in F0U0 (Figure 4.20).

With regards to soil NO$_3^-$-N, all urine treatments reached a peak of between 370 and 430 kg NO$_3^-$-N ha$^{-1}$ approximately one month following autumn urine application and two months following spring urine application. These peaks occurred slightly before the return of soil NH$_4^+$-N to a baseline of zero in the respective treatments. In all treatments where fertiliser was split applied, there were small peaks of up to 2 and 6 kg NO$_3^-$-N ha$^{-1}$ coinciding with the 200 and 400 kg N ha$^{-1}$ fertiliser application dates, respectively. In the F0U0 treatment, the model calculated zero soil NO$_3^-$-N.

### 4.3.10 Soil water

The volumetric soil water content at saturation (SAT), the drained upper limit (DUL), and the lower limit for plant uptake (LL) are shown for the same depth intervals described above: 0-20, 20-100, 100-200 and 200-700 mm (Figure 4.22). In the top soil layer, the soil water content was above the DUL from 18 Apr 2011 to 22 Oct 2011, and in all the other depths, the soil water content was above the DUL from 23 Mar 2011 to Nov 2011. From the beginning of Nov 2011 to 25 May 2012, the soil water content remained below the DUL, with the exception of the high rainfall period between 15 Dec 2011 and 23 Jan 2012. Deeper in the soil profile, in the 100-200 and 200-700 mm depths, the soil water content tended to be lower in the spring urine treatments, and autumn urine treatments, compared to the nil urine treatments (Figure 4.22). This is most likely due to enhanced evaporative loss from the additional plant uptake in the urine treatments.
Figure 4.19  Soil urea simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha\(^{-1}\), respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note the Y axes differ.
Figure 4.20 Soil NH₄⁺-N simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹, respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note Y axes differ.
Figure 4.21 Soil NO$_3$--N simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha$^{-1}$, respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. Large arrows = urine+fertiliser applications; small arrows = split fertiliser applications. Note Y axes differ.
Figure 4.22  Soil water simulated by APSIM from 0-20, 20-100, 100-200 and 200-700 mm depths for all treatments from 21 Feb 2011 to 27 Aug 2012. F0, F2 and F4 denote fertiliser rates of 0, 200 and 400 kg N ha⁻¹, respectively; and U0, UA and US denote nil, autumn and spring urine applications, respectively. DUL is the water content at the drained upper limit; SAT is the saturation point; LL is the lower limit for plant uptake at 15 bars of pressure. Note the y axes differ.
4.4 Discussion

The experimental and modelled results were generally in good agreement. The exception was N$_2$O emissions, which the model substantially overestimated. Discrepancies between modelled and measured data occurred with respect to pasture yield, N uptake, NO$_3^-$-N leaching, drainage and N$_2$O emissions. These are discussed below.

It is important to note that although simulated outputs from models are not a true representation of the N dynamics in a pastoral system, there is also a degree of error in the replicated experimental data (Bennett et al., 2013). With reference to model validation, this implies that attempting to achieve an exact fit between modelled and measured data is not only difficult, due to the error associated with both, but nonessential for model performance evaluation.

The main areas of concern where the modelled data did not agree with the measured data included the pasture yield and N uptake, which was underestimated by the model during the autumn winter months in winter, and subsequently caught up in the spring/summer months. Also, NO$_3^-$-N leaching and drainage was slightly underestimated in autumn urine treatments. Finally, N$_2$O emissions were considerably overestimated in all the urine-affected treatments.

4.4.1 Pasture growth and N uptake

The patterns observed over time in the pasture DM yield and harvested N were very similar. By the end of the experiment, in all treatments except F2U0 and F4U0, the cumulative modelled yield and uptake data was within the range of the measured data uptake (Figure 4.5 and Figure 4.7). However, the model underestimated the autumn/winter pasture yield and N uptake and ‘caught up’ over the spring/summer months. In the case of the autumn urine treatments, this autumn/winter period was also characterised by a large increase (and overestimate) in modelled N$_2$O emissions and denitrification (Figure 4.13 and Figure 4.14). Much of the autumn/winter drainage occurred during this period, so soil moisture conditions would have been particularly favourable for denitrification. Denitrification ceased in the autumn urine treatments on around 1 Oct 2011 (Figure 4.13), about the same time the underestimation of plant growth and N uptake ceased (Figure 4.4 and Figure 4.6). It is likely that the overestimate of modelled denitrification following autumn urine application (Figure 4.13 and Figure 4.14).
reduced the soil mineral-N in the model, to such an extent that it contributed to the model’s underestimation of pasture growth and N uptake over the 2011 autumn/winter period.

After 1 Oct 2011, the model simulated no further N₂O emissions in the autumn urine treatments. This was because the modelled soil NO₃⁻-N peaks had returned to zero at this time (Figure 4.21) so there was no further NO₃⁻-N available for denitrification. This was not realistic; because even small amounts of non-urine associated denitrification would have occurred after this time, particularly following rainfall events. This cessation of modelled denitrification, along with a lack of soil NO₃⁻-N post 1 Oct 2011 suggests that any soil NO₃⁻-N formed in the model via mineralisation after this time, was immediately taken up by pasture (or immobilised), thus serving as a possible explanation for the plant growth and N uptake ‘catching up’ after 1 Oct 2011.

However, underestimation of pasture yield and N uptake in the autumn/winter 2011 period was also observed in the spring urine and nil urine treatments, where no urinary N had been applied, therefore there was no ample substrate availability for denitrification and the subsequent limitation of pasture N availability as suggested above. An alternative possibility for the underestimated winter pasture growth and N uptake is that the model’s lower temperature limit of 5°C (i.e. the temperature below which pasture growth ceases) was greater than that under the experimental growth conditions (average daily and minimum temperatures for the 2011 and 2012 winter periods were 10.8°C and 3.4°C, respectively).

Following autumn and spring urine applications, the model underestimated the initial urine associated pulse of pasture growth and N uptake (Figure 4.5 and Figure 4.7). This is possibly due to the fact that the maximum N content of pasture in the model due to luxury uptake (5% N accumulation with no further yield above 4% N) was lower than that measured in the experiment (up to 5.1 and 5.5% N in the autumn and spring urine treatments, respectively). Furthermore, as suggested above, modelled denitrification possibly reduced more soil NO₃⁻-N to N₂O than was measured in the experiment, leaving less NO₃⁻-N available for plant uptake thus contributing to the observed underestimation of plant growth and N uptake following urine application.

In the case of the spring urine treatments, the model overestimated the pasture growth from Dec 2011 to Mar 2012 (Figure 4.5 and Figure 4.7). This was likely due to the high
rainfall over this time coupled with warm summer temperatures (Figure 4.3). Modelled denitrification and N₂O production in the spring urine treatments ceased by 16 Jan 2012 (Figure 4.13) but the pasture yield and N uptake continued to increase suggesting that the modelled pasture was now out-competing the denitrifiers for the soil NO₃⁻-N. Furthermore, the high summer rainfall, warmer temperatures and longer daylight hours would have created optimal conditions for plant growth, making it a more successful competitor for NO₃⁻-N at this time.

Other possible contributing factors to under/overestimations of modelled pasture growth and N uptake in all treatments could include the appropriateness of the initial estimate of the soil N pools and/or higher or lower mineralisation and immobilisation rates of soil N calculated by the model, than occurred in the experiment. Also, error in the pasture growth module itself, or in the measured pasture data cannot be ruled out. One obvious difference between the experiment and the model is that the model assumes 100% pasture cover with a white rye/clover mix at initialisation, whereas weeds would have been present in the lysimeters and pasture composition tended to change depending on season, and the treatments (if any) applied. Another probable source of error is the pasture cutting height. Although the model accurately harvested pasture to 1600 kg DM ha⁻¹ residual each time, it is unlikely (though not impossible) that the same precision was achieved with the hand held clippers. As such, a small difference in the cut height of the pasture at the lysimeter scale can make a much larger difference to the pasture DM yield and N uptake on a kg ha⁻¹ scale.

4.4.2 Water and solute movement through soil

4.4.2.1 Drainage

The modelled cumulative drainage was within the range of the measured drainage, in all treatments, with the exception of the autumn urine treatments (F0UA, F2UA and F4UA) where drainage was slightly underestimated by the model (Figure 4.9). The simulation missed the first drainage event (15–40 mm) on 28 Mar 2011 (Figure 4.9). In some cases (e.g. the F0U0, F2U0, F0US and F2US treatments) this late commencement of drainage, calculated by the model, may explain why the simulated drainage was closer to the lower confidence interval, suggesting the initial conditions in the model may have differed from the experiment. However, in the case of the autumn urine treatments, simulated drainage was underestimated by around 100 mm (~10% of cumulative drainage), so the lag in the
onset of drainage wouldn’t account for all of this. Another possibility is that due to the lysimeters being zero tension, drainage had to build up a head at the bottom soil layer before breakthrough occurred. APSIM may have overestimated this head, explaining the later onset of drainage, yet the good estimate of the total cumulative drainage in most treatments. Additionally, bypass flow mechanisms under periods of heavy rainfall may have played a small role in any discrepancy between the modelled and measured drainage, as APSIM does not account for soil bypass flow mechanisms. However, these differences between the measured and modelled data are not considered to be sufficient to notably affect the calculated drainage.

4.4.2.2 Leached NO₃⁻-N

The model calculated NO₃⁻-N leaching to be nil in the non-urine treatments (F0U0, F2U0, and F4U0) (Figure 4.11). In the case of the F2U0 and F4U0 treatments, this was less than the measured NO₃⁻-N leaching losses, which ranged from < 1-6 kg NO₃⁻-N ha⁻¹, with a single outlier of 28 kg NO₃⁻-N ha⁻¹ in the F4U0 treatment (Figure 4.11). These measured values were within or below the range of previously reported NO₃⁻-N leaching from fertiliser affected pasture (Ledgard et al., 1999; Silva et al., 1999; Di and Cameron, 2002b, 2007). In the case of F0U0, all the measured data was ≤ the level of detection of the analysing equipment, and this was also the case for much of the measured data in F2U0 and F4U0, thus making the apparent underestimation by the model in these treatments a trivial concern.

Interestingly, a large proportion of the cumulative measured NO₃⁻-N in the nil urine treatments occurred within the first 0-450 mm drainage (Figure 4.11), raising the question of whether this NO₃⁻-N leaching was antecedent to treatment application. This is unlikely because of the pre-experimental drainage carried out prior to treatment application. The NO₃⁻-N leaching during this time could be a result of accumulated fertiliser from the first 3 applications as well as from mineralisation flushes from rewetting of the soil in mid-late April after a dry summer.

The model tended to underestimate NO₃⁻-N leaching from the autumn urine treatments with a clear underestimation of cumulative NO₃⁻-N in F0UA, slight underestimation in F2UA, and although the simulated cumulative NO₃⁻-N leached in F4UA was within the 95% confidence limits, it was still lower than the lowest measured replicate (Figure 4.11). In addition, the modelled peak NO₃⁻-N concentration in the leachate was also
underestimated in the autumn urine treatments (Figure 4.10). These underestimates of NO$_3^-$-N leaching after autumn urine could be due to the large modelled denitrification and N$_2$O emissions following autumn urine application, leaving less NO$_3^-$-N in the soil available to be leached. The same underestimates in modelled NO$_3^-$-N leaching were not observed in the spring urine treatments (Figure 4.11). This is probably because autumn urine was swiftly followed by the 2011 winter drainage period, with high soil moisture and favourable conditions for denitrification. Also, considerable modelled denitrification (~40-50%) occurred deeper (> 200 mm) in the soil (Figure 4.17), which is not likely to have occurred to such an extent in the experiment, so where soil NO$_3^-$-N at depth (> 200 mm) would have been leached with drainage in the experiment, much of it was denitrified in the model. Spring urine was not immediately followed by high rainfall so conditions were not as favourable for denitrification in the model, leaving more NO$_3^-$-N available for leaching.

Another possible explanation for the underestimation of cumulative NO$_3^-$-N leached in the autumn urine treatments could be that the initial penetrative depth of 150 mm reached uniformly by the applied urine in the model, was not representative of the initial depth urine N reached in the experiment. The distribution of urine in the soil following deposition varies but has been observed to travel as deep as 600-700 mm via preferential flow (Williams et al., 1990a; Williams et al., 1990b; Monaghan et al., 1999). Monaghan et al. (1999) measured the distribution of cow urine in the soil within 6 hours following deposition in different soil types and reported that preferential flow of urine below 200 mm depth occurred in 9 out of the 10 soils examined. Williams et al. (1990a) reported that under simulated urination events, up to 46% of the urine was lost beyond 150 mm; and Williams et al. (1990b) observed that up to 62% of urinary N moved through 150 mm soil cores via preferential flow. These results suggest that perhaps the initial urine depth should be deeper than 150 mm; however, on the other hand, the same study by Monaghan et al. (1999) reported that 63-73% of urinary N was in the top 100 mm of soil after 6 hours, followed by 14-23% at 100-200 mm depth, and Williams and Haynes (1994) measured over 50% of deposited urine above 50 mm.

These studies clearly show there is large variability in the initial depth of urine following deposition, and bearing in mind that APSIM does not account for preferential flow in the simulations, an initial depth of 150 mm seems like a reasonable midway point. A sensitivity analysis carried out by Snow et al. (2011) showed that the initial depth of urine
in an APSIM simulation affected the amount of NO₃⁻-N leached, with greater NO₃⁻-N leaching observed at greater initial urine depths. The effect of the initial urine depth on the current simulations should also be determined.

In all the spring urine treatments, the modelled cumulative NO₃⁻-N leached was within the 95% confidence interval of the measured data (Figure 4.11). Presumably, the initial depth of the applied spring urine in the model did not affect the NO₃⁻-N leached to the same extent because in the model and the experiment, the NO₃⁻-N was subject to greater plant uptake, microbial uptake and denitrification processes prior to any drainage occurring.

The lag between the measured and modelled NO₃⁻-N peaks in the spring urine treatments (Figure 4.10) is possibly due to differences in NO₃⁻-N adsorption onto soil colloids between the simulation and the experiment. Although NO₃⁻-N is highly soluble and mobile under drainage conditions, adsorption of NO₃⁻-N from soil solution by anion exchange sites onto colloidal surfaces has been observed in variable charged soils that contain kaolinite, iron and aluminium oxides and hydroxide, and allophane (Black and Waring, 1979; Rasiah et al., 2003). Close et al. (2003) also observed anion adsorption in a Horotiu soil, but with a Br⁻ tracer rather than NO₃⁻ anions. The NO₃⁻-N retention capacity of a soil depends largely on the anion exchange capacity (AEC) and typically occurs in soils with a low net negative charge (Black and Waring, 1979). After spring urine was applied, there was at least 3 months before the Dec 2011/Jan 2011 drainage events, so the NO₃⁻-N that had not been utilised by plants or immobilised was held in the soil until this time. It is possible that a greater amount of the experimental spring urine associated NO₃⁻-N was held in the soil than was calculated by the model. Alternatively, NO₃⁻-N may have been bypassed by the drainage water due to NO₃⁻-N residing in micropores and this was not accounted for in the simulation.

Another factor that may affect the simulated vs experimental NO₃⁻-N leached is the pasture cover and composition. As mentioned previously, at initialisation, pasture cover in APSIM was set at 100% with a ryegrass/clover mix. This value is subject to change over time in the model depending on a range of factors including temperature and plant available water. During the experiment, the pasture in some lysimeters became sparse at times and weeds appeared, particularly during summer. A single species composition was carried out on the lysimeters on 2 Jul 2012 which found that ryegrass covered over 80% (± 5.9%) of the lysimeter surface area in all treatments, with the majority of the
remaining area being covered by weeds (Figure 3.24). A single weed can shade and out-compete pasture from a considerable area on a lysimeter. This variability in pasture cover and composition can create variability in pasture nutrient uptake (there was plenty of water this particular summer) and subsequently affect the availability of soil NO$_3^-$-N to be leached. The value at which the pasture cover is set on day 1 would make little difference to the pasture cover over the duration of the experiment because it is calculated on a daily basis by the model for the duration of the experiment.

In both the autumn and spring urine treatments, it appeared the onset of NO$_3^-$-N leaching occurred sooner in the simulation (at ~150 mm drainage) than in the experiment (~200 mm drainage) (Figure 4.10). This was probably due to the lag in the onset of drainage in the model. When leached NO$_3^-$-N is plotted against time (data not shown) there is no difference in the onset of leaching between the simulation and the experiment.

### 4.4.3 Nitrous oxide emissions

The model considerably overestimated N$_2$O-N emissions in urine affected treatments both temporally (Figure 4.13) and cumulatively (Figure 4.14). In the case of autumn and spring urine treatments, modelled cumulative N$_2$O emissions were 5 and 2 times greater than the measured N$_2$O emissions, respectively. On the other hand, the model calculated nil N$_2$O emissions from the non-urine treatments, where up to 1.1, 1.7 and 2.0 kg N$_2$O were measured from the F0U0, F2U0 and F4U0 treatments, respectively (Figure 4.14).

The measured cumulative N$_2$O results were within the range of emissions from pastoral soils treated with fertiliser and/or urine reported by other studies (von Rheinbaben, 1990; Di and Cameron, 2008; Luo et al., 2008; Cameron et al., 2013). De Klein and Van Logtestijn (1994) estimated minimum total denitrification losses from urine affected soil to be higher than the measured values in the current lysimeter study, at 20-50 kg N ha$^{-1}$ yr$^{-1}$, yet this is a large range, and the modelled N$_2$O emission estimates are at the higher end of this range.

The model calculated zero N$_2$O-N emissions from the F0U0 and F2U0 treatments and 0.5 kg N$_2$O-N ha$^{-1}$ from the F4U0 treatment (Figure 4.14). Nitrous oxide emissions were measured throughout the experiment from these treatments, ranging from 0.5-1.1, 1.0-1.7, and 1.5-2.0 kg N$_2$O-N ha$^{-1}$ from the F0U0, F2U0 and F4U0 treatments, respectively (Figure 4.14).
Modelled N₂O emissions were largely dependent on the denitrification rate, which is calculated in APSIM as follows (Equation 4.6) (Thorburn et al., 2010):

\[ R_{\text{denit},i} = k_{\text{denit}} NO_{3,i} \times C_A,i \times F_{\text{moist},i} \times F_{\text{temp},i} \]  

Where \( R_{\text{denit}} \) = denitrification rate at the \( i \)th soil layer (kg N ha\(^{-1}\) d\(^{-1}\)), \( k_{\text{denit}} \) = the denitrification coefficient (= 0.0006), \( NO_3 \) = the amount of NO\(_3\)-N in the soil layer (kg N ha\(^{-1}\)), \( C_A \) = the active carbon present in the soil layer, and \( F_{\text{moist}} \) and \( F_{\text{temp}} \) = factors (scaled from 0-1) accounting for moisture and temperature limitations on denitrification, respectively. The calculation of active C in APSIM is described previously by Equations 4.4 and 4.5.

Denitrification occurs in APSIM once the drained upper limit (DUL, or field capacity) is reached in the soil (\( F_{\text{moist}} \) factor), and the rate increases as soil water approaches saturation. Denitrification rate also increases with increasing temperature to a maximum of 50°C (\( F_{\text{temp}} \) factor) (APSRU, 2012).

The N₂O emissions are then calculated by combining the denitrification predictions with the N₂:N₂O ratio of emitted gas using the model of Del Grosso et al. (2000) and takes into account NO₃⁻-N concentration in the soil, and heterotrophic CO₂ respiration (Equation 4.7).

\[ R_{N_2/N_2O} = F_r(NO_3^-/CO_2) \times F_r(WFPS) \]  

Where \( R_{N_2/N_2O} \) = the ratio of N₂/N₂O; \( F_r(NO_3^-/CO_2) \) = the ratio as a function of the electron donor to the substrate; and \( F_r(WFPS) \) = a disturbance specific multiplier to account for the water-filled pore space effect on the N₂/N₂O ratio (Del Grosso et al., 2000).

The N₂:N₂O ratio reflects the completeness of N reduction and amongst other variables, is a function of gas diffusivity (Del Grosso et al., 2000). For example, higher ratios are typically observed in intact clayey soils because lower diffusion rates increase the likelihood of anaerobic conditions and prolong the residence time of N₂O in the soil through slower diffusion rates, thus increasing the likelihood that it will be further reduced to N₂. Some studies also show that the N₂:N₂O ratio of emissions increases under an increasing readily available C source (Weier et al., 1993a; Dittert et al., 2005; Wachendorf et al., 2008).
The overestimate of modelled N$_2$O emissions is potentially a result of one or a combination of the following factors: (a) the modelled soil moisture dynamics and $F_{\text{moist}}$ factor; (b) the production of N$_2$O at depth in the soil profile; and (c) the availability of active C in the soil profile ($C_a$). Nitrous oxide emissions via nitrification are also discussed.

(a) Soil moisture dynamics

The soil water content calculated by APSIM tended to remain above the DUL during autumn and winter, for months at a time, then fall below the DUL during summer (with the exception of the high rainfall period in Dec 2011/Jan 2012) (Figure 4.22). However, the modelled cumulative drainage profile was generally within the 95% confidence interval of the mean experimental drainage profile, thus suggesting the total flux of water passing through the soil was sensible. These high soil water contents calculated by APSIM likely resulted in enhanced anaerobic conditions and thereby increased capacity for denitrification, in turn contributing to APSIM’s overestimation of N$_2$O emissions. Although soil water was not measured in the lysimeters over time, the extended periods where modelled soil water was calculated to be above the DUL is questionable. The Horotiu silt loam soil is a very free draining soil, and near-saturated conditions do not prevail, therefore it is considered unlikely that the soil water content remained above the DUL for the duration the model suggests.

In calculating soil water content in APSIM, the values of SAT (saturation), DUL (drained upper limit) and LL (lower limit for plant uptake) are used to create a soil water retention curve (along with a fourth value, the zero water content of oven dry soil) and from here the retention curve across the entire water range is calculated (Huth et al., 2012). Hydraulic conductivity is calculated by constructing a two-region conductivity function with user specification of DUL and $K_S$. DUL is the point at which drainage becomes a low nominal value ($K_{DUL}$) and $K_S$ is rate of drainage between saturation and DUL (Huth et al., 2012). Hydraulic conductivity is assumed to be 0.1 mm d$^{-1}$ at DUL. Drainage above DUL is calculated by a macropore function (significant only above DUL) resulting in the hydraulic conductivity reaching $K_S$ at saturation (Huth et al., 2012).
Therefore, the value of DUL relative to SAT, and LL affects the water retention curve and thereby the volumetric soil water content and water-filled pore space (WFPS) at any given time. The increased WFPS and reduced aeration as a result of the extended high soil water conditions would have been favourable for denitrification, thus potentially explaining the greater and longer duration of N$_2$O emissions following the autumn and spring urine applications calculated by the model. Furthermore it may also explain why N$_2$ was such a dominant denitrification product (Figure 4.16), because under prolonged anaerobic conditions, NO$_3$-N is more likely to be completely reduced to N$_2$.

The position of the DUL on the soil water retention curve also determines the position of $K_{DUL}$ on the hydraulic conductivity curve (Figure 4.23b) and therefore the slope of the line between $K_{DUL}$ and $K_S$. The higher the saturated hydraulic conductivity ($K_S$) is relative to $K_{DUL}$, the steeper the slope of this line, and therefore the faster the rate of drainage between DUL and SAT. Soil saturated hydraulic conductivity ($K_S$) is affected by structure and texture, where finer textured soils (e.g. clays) have a lower $K_S$ than coarsely textured soils (e.g. sand). Soil profile layering, earthworm activity and plant roots and tortuosity can also affect the $K_S$ of soil (McLaren and Cameron, 1996). Typical values for $K_S$ range from <0.36 mm h$^{-1}$ in fine textured, poorly drained soils up to >360 mm h$^{-1}$ in coarse textured soils (McLaren and Cameron, 1996). Reported values of $K_S$ for the Horotiu silt loam vary considerably, e.g. Ghani et al. (1996) measured an average $K_S$ of 575 ($\pm$176) mm h$^{-1}$ at 0-75 mm depth, and Singleton and Addison (1999) reported $K_S$...
values to range from 64 to 692 mm h\(^{-1}\) in un-trodden and usual grazed pasture over a range of 50 mm depth increments down to 250 mm.

Another parameter that potentially affects the rate of drainage is the soil matric potential at DUL. The soil matric potential arises as a result of the adhesion and capillary action of water within the soil matrix (McLaren and Cameron, 1996) and is the force that must be overcome by plants in order to take up water from the soil. The lower the matric potential of water in a soil, the greater the force that is required to remove that water from the soil. In APSIM, the matric potential at SAT and the LL are assumed at fixed values of -1 and -15000 cm, respectively. The DUL has a default value of -100 cm, however this can be adjusted. The most appropriate matric potential for a soil at the DUL ranges between -50 to -200 cm (McLaren and Cameron, 1996).

(b) Depth of N\(_2\)O production

The model calculated N\(_2\)O production throughout the entire soil profile depth with 50 and 40% of the total N\(_2\)O production occurring below 200 mm in the autumn and spring urine treatments, respectively. Furthermore, in the autumn urine treatments, N\(_2\)O production increased substantially at the lowest depth increment (Figure 4.17). The soil NO\(_3\)-N substrate was plentiful in the 200-700 mm depth during the period of N\(_2\)O production (Figure 4.21), particularly in the autumn urine treatments, however, it was considered that the general decline of active soil C with depth (Figure 4.18) as well as declining microbial populations with depth would result in declining denitrification and N\(_2\)O production with depth.

Although there is a dearth of understanding of denitrification and N\(_2\)O production processes in the subsoil, most studies that have investigated it have found that the denitrification potential and N\(_2\)O production decreases at greater soil depths (Jarvis and Hatch, 1994; Murray et al., 2004). However, studies have also demonstrated that there is denitrification potential in subsoils where there is a source of readily available C and/or N (Weier et al., 1993b; Jarvis and Hatch, 1994; Müller et al., 2004; Murray et al., 2004). Clough et al. (1999) incorporated denitrification substrates to 1 m soil cores at 800 mm depth and observed maximum N\(_2\)O production at the 80-1000 mm depth, thus suggesting that denitrification at depth is limited by C and N substrates rather than a lack of denitrifying organisms.
In the model, at > 200 mm depth, particularly following autumn urine, N₂O production was generally not limited by moisture (F_{mois}), temperature (F_{temp}) (temperatures are more constant at depth and not so affected by surface temperature fluctuations), or a NO₃⁻-N substrate, therefore, active C (C_A) was likely the main limiting factor. This is discussed later.

An important assumption in the model that undoubtedly accounts for some of the model’s overestimation of N₂O is that all N₂O produced in the soil (at any depth) is immediately transferred to the soil surface and emitted to the atmosphere. This is a reasonable assumption for N₂O emissions that occur as a result of fertiliser applications, because the fertiliser remains near the soil surface, so there is little resistance for gas diffusion. However, the case is not the same for urinary derived N that has moved deeper in the soil profile and is subsequently denitrified, or naturally occurring background soil N that is denitrified deeper in the profile. Nitrous oxide that is produced in the soil profile may diffuse to the soil surface quickly or become entrapped and either eventually diffuse to the soil surface over time, be further reduced to N₂ or dissolved in soil water and leached through the soil profile (Samson et al., 1990; Clough et al., 2000; Clough et al., 2005). Increases in the WFPS and denitrification rates may increase entrapment of N₂O (Clough et al., 2005). Some ¹⁵N studies have quantified N₂O entrapment, e.g. Clough et al. (1999) demonstrated that 5.7% of ¹⁵N occurred as N₂O entrapped in 1 m soil columns and that the N₂:N₂O ratio increased as N₂O moved up the soil profile; and Clough et al. (2001) released entrapped gasses by destruction of soil cores and recovered 9.3% of the applied ¹⁵N as N₂O and 13.3% as N₂. It is difficult to determine how much of the discrepancy between the modelled and measured N₂O data can be attributed to this assumption because of the heterogeneity of soil physical properties across all the lysimeters.

(c) Active carbon content in the soil

The amount of available active C (Equation 4.4 and 4.5), and soil NO₃⁻-N in the different soil layers affects the modelled denitrification rate and subsequent N₂O evolution (Equation 4.6). While modelled soil NO₃⁻-N was plentiful, denitrification occurred and N₂O emissions were high and possibly unlimited, suggesting that active C was also unlimited in the model. Nitrate-N leaching and drainage profiles in the model were generally within the 95% confidence interval of the measured data, thus suggesting the modelled soil NO₃⁻-N was a good estimate. This suggests that of the two substrates,
active C may have been greater in the model than in the in-situ experiment and potentially contributed to the overestimation of N\textsubscript{2}O emissions. It is also possible that the slight increase in soil active C from 500-700 mm (Figure 4.18) contributed to the overestimate of N\textsubscript{2}O emissions.

Jarvis and Hatch (1994) demonstrated that the potential for denitrification in soils of up to > 6 m depth was enhanced by the addition of available C, and Weier et al. (1993b) found denitrification was stimulated by glucose addition down to 1.15 m. Later work by Murray et al. (2004) corroborated these findings but found that in a free draining loamy soil, only simple carbohydrates (i.e. glucose) could be utilised by the microbial population at depth (700-900 mm). Pre-experimental soil analysis down to 60 cm on the field trial site described in Chapter 5 (results not shown) indicated that soil microbial biomass N was greatest in the top 5 cm, declining with depth. Below 20 cm, the microbial biomass N was < 10 µg N g\textsuperscript{-1} soil, and at 60 cm depth it was ~2 µg N g\textsuperscript{-1} soil. These studies indicate that although the relative microbial population is smaller at depth compared to the more organic topsoil, the incorporation of a limiting substrate can stimulate the activity of the resident microbes at depth and potentially contribute to a significant amount of the soil’s total N\textsubscript{2}O production.

(d) N\textsubscript{2}O emissions via nitrification

Measured N\textsubscript{2}O emissions were also observed that were not associated with rainfall events (Figure 4.3), suggesting N\textsubscript{2}O evolution via nitrification may have occurred during the experiment. Production of N\textsubscript{2}O via nitrification can be substantial under aerobic soil conditions and when soil NH\textsubscript{4}\textsuperscript{+}-N is high (Bremner and Blackmer, 1978). Various studies have reported nitrification-derived N\textsubscript{2}O emissions to substantially contribute to the total N\textsubscript{2}O emissions from agricultural soils (Pihlatie et al., 2004; Uchida et al., 2012; Morse and Bernhardt, 2013). However, this is not exclusively the case, as some studies have shown the reduction of NO\textsubscript{3}\textsuperscript{-}-N as the dominant N\textsubscript{2}O producing mechanism in aerobic soils (Müller et al., 2002; Wolf and Brumme, 2002; Müller et al., 2004). A possible reason for this in grassland systems characterised by high N cycling rates, is a high rate of NO\textsubscript{2}\textsuperscript{-} oxidation, preventing the accumulation of NO\textsubscript{2}\textsuperscript{-} and thereby the production of N\textsubscript{2}O via nitrification (Müller et al., 2004). On the other hand, NO\textsubscript{2}\textsuperscript{-} accumulates via nitrification at least to some extent in soil following fertiliser application and urine deposition, and Venterea (2007) determined in a laboratory study that N\textsubscript{2}O production via
NO$_2^-$ in aerobic conditions was largely influenced by soluble and total C, total N and pH. Despite this, further understanding is required about the kinetic and biochemical controls of N$_2$O production under aerobic conditions in soil (Venterea, 2007).

In APSIM, N$_2$O emissions via nitrification are estimated as a fraction ($k_2$) of the nitrification rate (Equation 4.8). The default value of $k_2$ in the APSIM base simulation was mistakenly set at zero; however, normally this value is set at 0.002. This fraction has been reported to range from 0.001 to 0.05 and depending on soil and environmental conditions, is largely dependent on the rate of NO$_3^-$ production (Goodroad and Keeney, 1984).

\[
N_2O_{NIT} = k_2 \times Rate_{NIT} \tag{4.8}
\]

Where $N_2O_{NIT}$ is the amount of N$_2$O produced via nitrification (kg N$_2$O-N ha$^{-1}$ day$^{-1}$); $k_2$ is the proportion of nitrified N emitted as N$_2$O (value = 0.002); and $Rate_{NIT}$ is the nitrification rate (kg N ha$^{-1}$ day$^{-1}$). Goodroad and Keeney (1984) reported that under relatively constant temperature, and dry soil conditions, the production of N$_2$O is largely proportional to the production of NO$_3^-$-N (nitrification) and that 0.1-0.2% is emitted as N$_2$O.

The $k_2$ value of zero in the current APSIM simulation is not correct, however, the default value of 0.002 is at the higher end of the scale reported by Goodroad and Keeney (1984), which is not necessarily erroneous, given that previous work on N$_2$O emissions via nitrification is varied and in some cases contradictory. As such it is suggested this parameter is investigated in the current simulation even though there is already an overestimate of N$_2$O emissions.

4.4.4 Soil inorganic N

The modelled soil NH$_4^+$ content peaked within a few days following urine applications, but didn’t return to background levels until around 3 months following urine application in the autumn and spring urine treatments (Figure 4.20). Following fertiliser and urine applications to soil, the N does not usually remain in the NH$_4^+$ form for this long, as it is further nitrified to NO$_3^-$. In other studies (both laboratory and field based) where urine has been applied to soil, NH$_4^+$ is completely nitrified in up to 55 days e.g. Bertram et al. (2009), Clough et al. (2009) and Orwin et al. (2010). In the field trial described in Chapter 5 of this document, on the same soil type under similar temperatures, soil NH$_4^+$
concentrations in the top soil peaked within 1-2 days following urine application, and returned to background levels 21 days following urine application (Figure 5.24a).

In APSIM, ‘potential nitrification’ in the SoilN module is calculated using Michaelis-Menton kinetics. The rate-limiting part of the nitrification process, i.e. the conversion of NH$_4^+$ to NO$_2^-$ is represented using a ‘maximum reaction velocity’ (Vmax) and an NH$_4^+$ concentration at $\frac{1}{2}$ Vmax (Km) (Equation 4.9) (Meier et al., 2006; APSRU, 2012):

$$\text{Potential rate} = \frac{V_{\text{max}} \times NH_4(ppm)}{NH_4(ppm) + Km}$$

Where potential rate = the potential nitrification rate, Vmax = the maximum reaction velocity for nitrification (µg N kg$^{-1}$ soil d$^{-1}$), $NH_4(ppm)$ = the NH$_4^+$ concentration in soil (µg g$^{-1}$ soil) and Km = the NH$_4^+$ concentration at $\frac{1}{2}$ Vmax. The daily nitrification is then calculated from the potential nitrification rate accounting for sub-optimal water, temperature and pH conditions.

The rate of modelled nitrification might also partially explain the underestimated pasture growth and N uptake immediately following urine deposition (Figure 4.4 and Figure 4.6), and also the underestimated NO$_3^-$-N leached in the autumn urine treatments (Figure 4.11) where the NH$_4^+$ ions were held by the soil resulting in lower leaching of NO$_3^-$-N than was measured in the experiment.

**4.4.5 Validation discussion summary**

- Modelled cumulative pasture growth and N uptake was generally within the range of the measured data; however, the modelled pasture growth and N uptake was underestimated during the winter months. It is suggested that the large amounts of modelled denitrification potentially played a role in these underestimations, and also, the lower temperature limit for pasture growth in the model may have differed from that during the experiment.

- The first measured drainage event was not measured by the model. Cumulative NO$_3^-$-N leaching was underestimated in the autumn urine treatments (with the exception of F4UA), which could potentially be attributable to the initial depth of urine application and also the large amounts of denitrification estimated by the model.
Nitrous oxide emissions were considerably overestimated by the model in all the urine affected treatments. It is suggested that this could be due to an overestimation of the models estimate of the volumetric soil water content and an underestimation of the saturated hydraulic conductivity in the model. Other possible contributing factors include the relatively large N₂O production at depth (> 200 mm) and the model’s calculation of active soil C throughout the soil profile (but particularly at depths >200 mm). Furthermore, the model’s assumption that gas evolved in the soil is emitted immediately will contribute to the overestimation of modelled N₂O. The coefficient for N₂O production via nitrification was zero in the model. Although this value is not realistic, contradicting results in the literature make it difficult to determine the most suitable value. Nevertheless, the parameter/s responsible for this output should be checked.

In conclusion, the current APSIM simulation is not considered suitable to carry out extrapolative simulations to predict the fate of applied urinary and fertiliser N in different climatic regions and/or different soil types. The main reason for this is the large overestimation of N₂O emissions. Although the model slightly underestimated NO₃⁻-N leaching in the autumn urine treatments, missed the first drainage event and underestimated pasture N uptake in winter, these were all relatively minor incongruities with modelled cumulative values within (or just outside in the case of leached NO₃⁻-N) the 95% confidence interval of the measured data. The modelled N₂O emissions were well above the 95% confidence interval of the measured data, and previously reported data, therefore some parameter adjustments may be required in the model.

The key parameters and/or input values identified as potentially contributing to the incongruities between the modelled and measured data included:

(i) depth of urine deposition;
(ii) the difference between DUL and saturation (SAT);
(iii) the difference between DUL and the lower limit for plant uptake (LL15);
(iv) saturated hydraulic conductivity (Kₛ);
(v) soil matric potential at DUL;
(vi) soil active C content;
(vii) the lower temperature limit for pasture growth;
(viii) maximum luxury N uptake by pasture;
(ix) potential nitrification;
(x) the denitrification coefficient ($k_{\text{denit}}$);
(xi) coefficient for the ratio of $\text{N}_2:\text{N}_2\text{O}$ produced; and
(xii) the nitrification coefficient for $\text{N}_2\text{O}$ production ($k_2$).

An exploratory sensitivity analysis was performed with the aim of generating a greater understanding of the effects that adjustments to the parameter and/or input values had on the simulated fate of $\text{N}$ under the same experimental conditions. The intention of the sensitivity analysis was not necessarily to improve shortcomings in APSIM’s current parameter values using only the single set of data generated from the lysimeter experiment (Chapter 3) (although improvements cannot be ruled out). Rather its purpose was as an investigative exercise to isolate modules or parameters which may require further validation or parameterisation for the best possible simulation of pastoral systems using APSIM.

This sensitivity analysis does not contribute to answering the objectives of this thesis; therefore it is included as additional information in Appendix B.
Chapter 5
What is the ‘effective area’ of a urine patch?

5.1 Introduction

The deposition of nitrogen (N) from grazing animals plays a key role in the cycling of N in pastoral systems (Haynes and Williams 1993). Urine deposition accounts for nearly 70% of the N returned to pasture at rates up to 1000 kg N ha\(^{-1}\), which results in the distribution of easily identifiable patches of tall, dense, dark green pasture (Ledgard \textit{et al.}, 1982; Steele, 1982).

Urine patches are estimated to cover between 20-30% of the grazed paddock area per year, depending on the stocking rate (Silva \textit{et al.}, 1999; Moir \textit{et al.}, 2011). The literature suggests dairy cows urinate a volume of approximately 2 L (Doak, 1952), 10 to 12 times per day (Jarvis \textit{et al.}, 1995), covering an average surface area of 0.26 m\(^2\) (Petersen \textit{et al.}, 1956; Davies \textit{et al.}, 1962; Hogg, 1968; Richards and Wolton, 1976), that ranges from 0.16–0.49 m\(^2\) (Haynes and Williams, 1993). These values vary significantly, and are influenced by grazing conditions and many other environmental factors (Haynes and Williams, 1993). The volume of soil wetted by a urine patch varies with surface area, soil moisture, surface water repellence, surface compaction, microtopography, vegetation cover, slope and wind (Williams and Haynes, 1994).

Of the urinary N that is returned to the soil, some is volatilised as NH\(_3\), but much is eventually nitrified resulting in the accumulation of nitrate (NO\(_3^-\)-N). Pasture N uptake from a urine patch has been estimated at between 300 and 700 kg N ha\(^{-1}\) yr\(^{-1}\) (During and McNaught, 1961; Ball \textit{et al.}, 1979; Ledgard, 2001), therefore, with urine N concentrations of up to 1000 kg N ha\(^{-1}\) yr\(^{-1}\), the N loading from a urine patch far exceeds plant demand. Surplus NO\(_3^-\)-N remaining in the soil is vulnerable to leaching, and this can be a considerable N loss pathway from dairy farming systems (Silva \textit{et al.}, 1999).

As discussed in Chapter 2, NO\(_3^-\)-N leaching can result in nutrient enrichment and accelerated eutrophication of surface waters (Galloway \textit{et al.}, 2004). These environmental impacts have become a major global concern, and much research in New Zealand is currently focused on reducing and mitigating the effects of NO\(_3^-\)-N leaching from dairy farming systems.
A urine patch can be considered as comprising a ‘wetted area’ (where urine is directly voided) and an ‘effective area’ which also includes an area outside the wetted area (as well as the wetted area itself) that can access urinary N through plant root extension and N diffusion through the soil (Lantinga et al., 1987; Tinker and Nye, 2000). The effective area reported in the literature for cattle ranges from 0.03–1.1 m² (Lotero et al., 1966; Moir et al., 2011), however, data are very limited. Decau et al. (2003) measured plant uptake from two zones outside a 0.4 m² urine patch within lysimeters and found that urinary N did not diffuse beyond 20 cm from the edge of the urine patches. Lotero et al. (1966) measured plant response to urine N in concentric bands around the centre of existing urine spots and found that DM yield decreased linearly from the centre to the periphery of the urine patch, affecting a total of 0.9-1.2 m². Moir et al. (2011) observed seasonal variation in the effective area whereby urine affected areas tended to be larger from spring/summer deposited urine, and smaller in winter and autumn. These differences were mainly attributed to rapid winter NO₃⁻-N leaching when soils are draining (less N available for plant uptake) but other factors may include animal N intake in feed (less feed in winter), animal water intake (less water ingested in winter therefore lower urine volumes), and higher spring/summer pasture growth rates (Moir et al., 2011).

Many N leaching studies have used lysimeters to quantify NO₃⁻-N losses from urine patches (Clough et al., 1996; Clough et al., 1998b; Silva et al., 1999; Di and Cameron, 2002a; Decau et al., 2003; Decau et al., 2004; Di and Cameron, 2004; Di and Cameron, 2007). In many cases, where lysimeter size is 500 mm diameter by 700 mm depth or smaller, urine treatments tend to cover the whole area of the lysimeter. However, a lysimeter is a confined area of soil, and its casing represents a physical barrier to the surrounding soil. Therefore urine patch leaching data obtained under such conditions are only reflective of the wetted area of a urine patch and do not account for the effective area. This suggests that urine associated N leaching from such lysimeter studies could be overestimated when compared to what would be truly lost in an identical field scenario.

Agricultural systems models (e.g. Overseer® and APSIM) are tools used to estimate N losses from grazed pasture systems (e.g. Di and Cameron (2000)) and are increasingly relied upon as decision-support tools by regional councils, industry and farmers. Many of these models are parameterised with data from lysimeter studies to improve their simulation capabilities and for model validation. However, if the observed data from lysimeter studies has not accounted for urine patch edge effects, then the models cannot
accurately do so either. More quantitative research is therefore needed to measure the significance of potential edge effects of urine patches so that this information is accurately represented in agricultural systems models and subsequent nutrient budgets and N leaching estimates.

This chapter presents data from a field trial where $^{15}$N amended urine and natural abundance urea fertiliser treatments were applied concurrently, and both the wetted area of the urine patch, and the surrounding area were monitored. The objectives of this study were to quantify the extent of the ‘effective area’ of a spring deposited urine patch in combination with spring urea fertiliser application, on both the pasture response and soil N dynamics. The hypotheses tested were as follows:

a) a significant proportion of urinary N will be taken up by pasture outside the wetted area of a urine patch;

b) fertiliser N applied concurrently with a urine deposition event will be surplus to plant requirements, and therefore will not result in increased pasture growth within the effective area of a urine patch.

c) soil N and microbial biomass N will increase outside the wetted area of a urine patch;

### 5.2 Methodology

#### 5.2.1 Experimental setup

A plot trial was undertaken at Scott Farm, a dairy farm owned and operated by Dairy NZ, and located near Hamilton in the Waikato Region of New Zealand (latitude -37.7°S, longitude 175.4 °E). The soil type at the trial site was a Horotiu silt loam (Typic Orthic Allophanic soil) (Hewitt, 1998). Prior to beginning the field trial, 12 soil cores (0-7.5 cm) were taken randomly from the site, bulked and analysed for basic soil properties. These soil properties are shown in Table 5.1.
Table 5.1 Key soil properties (0-7.5 cm depth) at the field site in August 2011.

<table>
<thead>
<tr>
<th>Soil Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.8</td>
</tr>
<tr>
<td>Organic C (g kg(^{-1}) soil)</td>
<td>74</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}) soil)</td>
<td>6.5</td>
</tr>
<tr>
<td>Olsen P (µg mL(^{-1}))</td>
<td>47</td>
</tr>
<tr>
<td>Sulphate sulphur (g kg(^{-1}) soil)</td>
<td>0.051</td>
</tr>
<tr>
<td>Potassium (cmol(_c) kg(^{-1}))</td>
<td>0.38</td>
</tr>
<tr>
<td>Calcium (cmol(_c) kg(^{-1}))</td>
<td>5.4</td>
</tr>
<tr>
<td>Magnesium (cmol(_c) kg(^{-1}))</td>
<td>1.20</td>
</tr>
<tr>
<td>Sodium (cmol(_c) kg(^{-1}))</td>
<td>0.12</td>
</tr>
<tr>
<td>Cation exchange capacity (cmol(_c) kg(^{-1}))</td>
<td>21</td>
</tr>
<tr>
<td>Total base saturation (%)</td>
<td>34</td>
</tr>
</tbody>
</table>

The trial area was fenced off to prevent access by grazing animals and regularly cut for four months prior to the commencement of the experiment. At this time, any visible dung patches were also removed. In early October 2011, there had been insufficient late winter rain to remove any previous urine patch effects, therefore the trial area was irrigated, with 350 mm of water over a period of 4 days (Friday 7\(^{th}\) October 2011 – Monday 11 October 2011) using K-line irrigator pods.

The plot designs were circular and consisted of three annular rings (see Figure 5.1). The treatments (urine and/or fertiliser) were applied to the central ring, which represented the ‘wetted area’ and this plus the two outer rings represented the ‘effective area’. A review of the literature showed the average area covered by a cattle urine patch is 0.26 m\(^2\), therefore the centre ring of each plot had a radius of 0.3 m (area 0.28 m\(^2\)). The second and third annular rings around the central ring both extended an additional 0.25 m radius with areas of 0.67 and 1.06 m\(^2\), respectively (Figure 5.1). A stainless steel template was constructed (Figure 5.2) to aid with accurate treatment application and for delineation sampling.
5.2.2 Treatments

Treatments included a control (U0F0), a fertiliser only treatment (U0F+), a urine only treatment (U+F0) and a urine plus fertiliser treatment (U+F+). The central ring of the plots (referred to henceforth as “Zone A”) received the fertiliser and/or urine treatments, and the middle and outer rings (referred to henceforth as “Zone B” and “Zone C”, respectively) did not receive any treatment. There were four replicates and 16 plots arranged in a latin square. The treatments are detailed in Table 5.2 below and were applied on 17th October 2011.

<table>
<thead>
<tr>
<th>Treatment Name</th>
<th>Description</th>
<th>$^{15}$N Urine (kg N/ha/yr)</th>
<th>Fertiliser (kg N/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1   U0F0</td>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2   U0F+</td>
<td>Fertiliser only</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>3   U+F0</td>
<td>Urine only</td>
<td>800</td>
<td>0</td>
</tr>
<tr>
<td>4   U+F+</td>
<td>Urine + fertiliser</td>
<td>800</td>
<td>35</td>
</tr>
</tbody>
</table>

Fresh cow urine was collected during the morning milking from a dairy farm in Waharoa, Waikato on 16th October 2011 (the day before treatment application). The urine was
bulked and analysed for total N. It was standardised to a concentration of 11.2 g N L\(^{-1}\) and a \(^{15}\)N enrichment of 5 atom% was attained by concentrating N with natural abundance urea and 99 atom% \(^{15}\)N enriched urea. Two litres of urine were applied to Zone A at a rate of 800 kg N ha\(^{-1}\). The urine concentration and volume were selected based on previous estimates of average New Zealand pasture based dairy cow urination events (see Sections 2.3.1 and 2.3.2). The urine was applied slowly using a watering can to ensure that urine did not run off outside Zone A. Granulated natural abundance urea fertiliser was applied evenly over the Zone A area at a rate of 35 kg N ha\(^{-1}\) after urine application. This fertiliser rate was based on a common fertiliser dressing rate for a dairy system in the Waikato region. The urine treatments were washed in with 2 L of water (equivalent to 10 mm rainfall) to prevent pasture scorching. All other plots (including fertiliser only treatments and controls) also received 2 L water to ensure soil moisture was the same across all plots.

5.2.3 Monitoring regime

Measurements and monitoring began on 18\(^{th}\) October 2011. A co-variate pasture cut was carried out on 17\(^{th}\) October prior to the application of treatments. The sampling regime included collecting pasture yields and cuttings, and soil core samples. Samples (both herbage and soil) were collected from within each annular ring (Zones A, B and C) within each plot. To ensure the plots were sampled representatively, each zone was divided evenly into six segments. Two segments were designated for soil coring, two for pasture yields, and two for taking pasture cuttings. The samples from the two segments within each annular ring were bulked together. The purpose of designating segments within each zone, in each plot, was two-fold; (a) to reduce spatial variability within each plot, and (b) to prevent anomalies in herbage yield measurements due to soil coring damage. The above-mentioned circular, stainless steel template outlined the segments and was used as a guide during sample collection.

Daily weather data was obtained over the duration of the experiment from the Ruakura climate station (via the NIWA Virtual Climate Station dataset (Tait and Turner, 2005)), located approximately 7 km from the field site.
5.2.4 Pasture yield collection and analysis

Pasture was cut down to a height of 3 cm with electric clippers, powered by a generator, leaving a residual of 1600 kg DM ha⁻¹. Pasture was cut every 21-28 days in accordance with a typical Waikato dairy grazing regime. The grass clippings were placed in paper bags and oven dried at 50°C for 48–72 hours. The dried pasture was weighed and recorded, after which, it was ground using a Cyclone Sample Mill equipped with a 1 mm screen (UDY Corporation, USA). To minimise the risk of cross contamination, plots that did not receive ¹⁵N amended urine were always cut and ground first, and equipment was washed after use.

The samples were analysed for total N and ¹⁵N on an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20). A description of the IRMS analysis procedure is detailed in Section 3.2.8. The ¹⁵N recovered from pasture, as a percentage of the total ¹⁵N applied as urine, was then calculated using Equation 5.1 (Cabrera and Kissel, 1989):

\[
¹⁵N_{Recovered} = \frac{100}{1} \times \frac{p \times (c - b)}{f \times (a - b)}
\]  

\[5.1\]
Where \( p \) = the moles of N in the sample; \( f \) = the moles of N in the \(^{15}\)N enriched urine applied; \( c \) = atom % of the sample; \( a \) = atom % of the \(^{15}\)N enriched urine applied (5.0 atom %); and \( b \) = atom % \(^{15}\)N abundance of the control.

### 5.2.4.1 Pasture cuttings: collection and analysis

Pasture cuttings were collected within the first two months of the experiment to understand in greater detail, the initial uptake of \(^{15}\)N in the herbage at more regular intervals than the yield data permitted. Cuttings were collected using purpose built grass cutters on the following dates: 18 Oct 2011 (day 1); 21 Oct 2011 (day 4); 25 Oct 2011 (day 8); 28 Oct 2011 (day 11); 7 Nov 2011 (day 21); and 29 Nov 2011 (day 43). The clippings were placed into paper bags and oven dried at 50°C for 48–72 hours, weighed and then ground using the Cyclone Sample Mill with 1 mm screen (UDY Corporation, USA). The ground cutting samples were analysed for total N and \(^{15}\)N on an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20).

### 5.2.5 Soil collection and analysis

Soil cores were collected to a depth of 20 cm, and cut into 2 depths: 0-7.5 cm and 7.5-20 cm. The number of cores able to be taken from each segment, particularly from a single segment in Zone A, was limited due to the small area available. Therefore, two cores were taken from Zone A (one core from each segment, bulked together); four cores were taken from Zone B (two from each segment, bulked); and six cores were taken from Zone C (three from each segment, bulked). Soil core samples were collected on the following dates: 18 Oct 2011 (day 1); 25 Oct 2011 (day 8); 31 Oct 2011 (day 14); 7 Nov 2011 (day 21); 29 Nov 2011 (day 43); 19 Dec 2012 (day 63); 31 Jan 2012 (day 106); and 19 Apr 2012 (day 185). The final soil sampling on day 185 was cored, from Zone A only, to a depth of 60 cm, and cut into three depths of 0-7.5 cm, 7.5-30 cm and 30-60 cm.

The field moist soil samples were placed in plastic bags and transferred in a chilly bin from the site to a chiller (< 4°C). Preparation of the soils for analysis began within 24 hours of collection. Firstly, the soil samples were passed through a 4 mm sieve and any herbage material was removed.

### 5.2.5.1 Gravimetric soil moisture content

Sub-samples of field moist sieved soil (5-10 g) were weighed into tin cups of a known weight, and oven dried at 105°C for 24 hours. The samples were cooled in a desiccator,
then re-weighed and the gravimetric water content was calculated using Equation 5.2 (Topp and Ferré, 2002):

\[ \theta_g = \frac{M_w}{M_s} \]  

Where \( \theta_g \) = the gravimetric soil moisture content (g water per g oven dry soil); \( M_w \) = the mass of water (g) \((\text{mass of field moist soil (g)} - \text{mass of oven dry soil (g)})\) and \( M_s \) = the mass of oven dry soil (g).

### 5.2.5.2 Soil inorganic nitrogen

For the first four soil collections, the field moist equivalent of 5 g oven dry soil (approximately 7-8 g field moist soil) was extracted with 25 mL 0.5 M potassium sulphate (K\(_2\)SO\(_4\)) at 20°C for two hours on a reciprocating shaker (Mulvaney, 1996). The samples were then centrifuged for 5 minutes at 3660 rpm. From sample collection #5 (29 Nov 2011) onwards, 10 g of field moist equivalent soil was weighed into conical flasks and extracted with 50 mL K\(_2\)SO\(_4\). The extractant was then filtered through fluted 11 cm Whatman #42 filter papers into 50 mL screw cap specimen vials and frozen until analysis. Blanks consisting of filtered K\(_2\)SO\(_4\) were also prepared. The extracts were analysed for NH\(_4\)^+-N and NO\(_3\)^--N + NO\(_2\)^--N on a Skalar SAN++ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). The method for NH\(_4\)^+-N analysis is based on the modified Berthelot reaction (Rhine et al., 1998) where NH\(_3\) is chlorinated to monochloramine which reacts with salicylate and is then oxidised to form a blue/green coloured complex which is measured colourimetrically at 660 nm. Equation 5.3 was used to determine the inorganic N concentrations in the soil:

\[ N_s = \frac{N_e \times V}{M_s} \]  

Where \( N_s \) = the inorganic N content (mg kg\(^{-1}\) dry soil); \( N_e \) = the inorganic N concentration of soil extract (mg L\(^{-1}\)); \( V \) = the volume of solution (K\(_2\)SO\(_4\) extract + soil moisture) (L) and \( M_s \) is the mass of oven dry soil (kg).

### 5.2.5.3 \(^{15}\)N diffusion of soil inorganic nitrogen

The non-fumigated K\(_2\)SO\(_4\) extracts were analysed for NH\(_4\)^+-\(^{15}\)N and NO\(_3\)^--\(^{15}\)N using the diffusion methodology described by Brooks et al. (1989). A minimum of 40–50 µg of N was required for the analysis of \(^{15}\)N, therefore the concentration of NH\(_4\)^+-N and/or NO\(_3\)^--N...
N, and volume of extract available dictated which samples could be prepared for diffusion. Thus, only samples with NH$_4^+$-N and/or NO$_3^-$-N concentrations > 4.0 µg N mL$^{-1}$ were able to be processed for inorganic $^{15}$N diffusion.

The diffusion method involved piercing 7 mm diameter discs of Whatman GF/D filter papers with 80 mm lengths of stainless steel wire. These were then spring loaded across the inside of 100 mL container screw cap lids and 10 µL of 2.5 $M$ KHSO$_4$ was pipetted onto the filter discs. These were set aside for no longer than 10 minutes to minimise any potential air contamination. Aliquots of non-fumigated K$_2$SO$_4$ extract containing the equivalent of 40 µg of N, were placed in 100 mL containers along with a 4 mm glass bead.

For $^{15}$N-NH$_4^+$ analysis, 0.2 g of furnace dried MgO, was added (to raise the pH of the solution), and then the lid with the spring-loaded acidified filter paper was screwed tightly down. The container was then carefully but thoroughly mixed. For NO$_3^-$-$^{15}$N analysis, 0.2 g of MgO was added, and the containers were left uncovered to allow volatilisation of any NH$_4^+$-N in the sample. The filter paper discs were then acidified and 0.4 g of Devarda’s alloy was added, before quickly applying the lid, and mixing the solution. Mixing was important to ensure the pH was raised, and complete reduction of NO$_3^-$-N to NH$_4^+$-N occurred, but care was taken to prevent the alkaline solution from contacting the acidified filter paper and neutralising the acid. The containers were left at room temperature (≈20°C) for 6 days without further mixing.

The wires were then removed and, with the filter discs were dried in an oven at 35°C. The filter papers were then transferred into tin capsules. The tin capsules were folded up and placed in an auto-analyser tray and sealed shut with tape until analysis. The filter paper discs were analysed for $^{15}$N and total N by direct combustion by Isotope Ratio Mass Spectrometry (EA-CF/IRMS; PDZ Europa GSL / 20-20). A description of the IRMS analysis procedure is detailed in Section 3.2.8. The $^{15}$N recovered from the soil inorganic fraction, as a percentage of the total $^{15}$N applied as urine, was then calculated using Equation 5.1.

### 5.2.5.4 Soil microbial biomass N analysis

The chloroform fumigation extraction method (Brookes et al., 1985) was used to determine soil microbial biomass N. Normally, the fumigation extraction method involves duplicate sub-samples of field moist soil being weighed out, one set for
fumigation, and the other, non-fumigated set for immediate extraction with 0.5 M K₂SO₄. However, because 0.5 M K₂SO₄ extractant was used for the determination of soil inorganic N, these were essentially “non-fumigated” extracts, therefore only the fumigated extracts required preparation.

Firstly, chloroform was purified, by shaking 100 mL of chloroform (Analar grade) with 200 mL of DI water in a 500 mL separating funnel. The lower water layer was discarded and the process was repeated twice more. The purified chloroform was stored in a stoppered glass bottle with anhydrous Na₂SO₄. Purification is required because commercial grade chloroform contains ethanol as a stabiliser, which is a C source (Jenkinson et al., 2004).

Sub-samples of field moist soil (5 g oven dry equivalent soil from collections 1-4; and 10 g oven dry equivalent soil from collections 5-8) were weighed into aluminium cups, which were then positioned inside a desiccator along with a small conical flask containing 25 mL purified chloroform and ~5 boiling chips. The desiccator lid was secured and it was evacuated using a vacuum pump until the chloroform was boiling (indicated by vigorous bubbling), at which point it was sealed, the vacuum was removed, and it was left inside a dark cupboard for at least 12 h. After this time, the desiccator was brought back to atmospheric pressure and the flask with any remaining liquid chloroform was removed. The desiccator was then re-evacuated and flushed three times with fresh air and left open inside the fume cupboard for 0.5 h to remove any remaining chloroform vapour. The soil samples were then removed and re-weighed (the fumigation process can slightly dry out the samples), after which they underwent extraction with 0.5 M K₂SO₄ as described in Section 5.2.5.2. The extracts were analysed for NH₄⁺-N and NO₃⁻-N + NO₂⁻-N on a Skalar SAN⁺⁺ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). Soil microbial biomass N was determined using the persulfate oxidation methodology described by Cabrera and Beare (1993). A peroxydisulfate (K₂S₂O₈) solution was used to oxidise the N in the K₂SO₄ extracts to NO₃⁻ under high temperature and alkalinity. Under these conditions, the K₂S₂O₈ decomposes according to Equation 5.4 below, generating the O₂ required for the N oxidation.

\[
K_2S_2O_8 + H_2O \rightarrow 2 KHSO_4 + \frac{1}{2}O_2
\]  

The oxidising solution was prepared by dissolving 100 g potassium persulfate (K₂S₂O₈), 60 g boric acid (H₃BO₃) and 15 g sodium hydroxide (NaOH) in 2 L of deionised water.
Aliquots of either fumigated or non-fumigated K$_2$SO$_4$ extracts (5 mL), and the oxidising reagent (5 mL) were pipetted into autoclave tubes and the lids were immediately applied. The samples were autoclaved at 120˚C for 30 minutes, allowed to cool, and then refrigerated until analysis. The samples were analysed the following day for NO$_3^-$-N and NH$_4^+$-N on a Skalar SAN++ segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands).

The oxidation process converted NH$_4^+$-N and organic forms of N in the K$_2$SO$_4$ extracts to NO$_3^-$-N. Nitrate-N already present in the extract was unchanged. Therefore, the organic N fraction was calculated (Equation 5.5) as the total NO$_3^-$-N value obtained from the analysis above less the previously analysed NO$_3^-$-N and NH$_4^+$-N (ppm).

\[
\text{Organic N} = \text{Total } N - \text{NO}_3^- N - \text{NH}_4^+ N  \tag{5.5}
\]

The microbial biomass N was calculated (Equation 5.6) as the flush of N extracted from a fumigated soil less that extracted from a non-fumigated soil:

\[
\text{Microbial Biomass N} = N_f - N_{uf}  \tag{5.6}
\]

Where \( N_f \) = the organic N from the fumigated soil extract and \( N_{uf} \) = the organic N from the non-fumigated soil extract.

The microbial biomass N was adjusted by a \( k_{EN} \) factor of 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996), which accounts for the efficiency of extraction of organic microbial N after fumigation.

### 5.2.5.5 Soil microbial biomass $^{15}N$

After analysis of the oxidised K$_2$SO$_4$ extract sub-samples, these solutions were prepared for $^{15}N$ diffusion using the same methodology (Brooks et al., 1989) as described above (Section 5.2.5.3). However, because the persulfate oxidation process described in Section 5.2.5.4 converts organic and NH$_4^+$ forms of N to NO$_3^-$-N, the diffusion method only needed to be carried out for NO$_3^-$-N. As with the inorganic $^{15}N$ diffusions (Section 5.2.5.3), a minimum of 40–50 \( \mu \)g of N was required for the analysis, therefore the concentration of NO$_3^-$-N in the K$_2$SO$_4$ extract sub-samples, and the volume of extract available, dictated which samples could be prepared for diffusion. Thus, only samples with NO$_3^-$-N concentrations > 4.0 \( \mu \)g N mL$^{-1}$ (in both the fumigated and non-fumigated extracts) were able to be processed for $^{15}N$ diffusion. Once the diffusion process was...
complete, the acidified filter paper discs were analysed for $^{15}$N and total N by direct combustion using an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20) (Section 3.2.8). The $^{15}$N recovered from the microbial biomass as a percentage of the total $^{15}$N applied as urine was then calculated using Equation 5.1. The calculated $^{15}$N recovery from the non-fumigated sample was then subtracted from the $^{15}$N recovery of the corresponding fumigated sample to give the total microbial biomass $^{15}$N recovery.

5.2.5.6 Soil total N analysis

All soils were oven dried at 50°C for 48 hours. A representative sub-sample was removed by riffling the dried soil. The sub-samples were ground using a RockLabs orbital soil grinding machine CH-4 (Gilco Products, Albany, Auckland, New Zealand) and analysed for total N and $^{15}$N atom % on an Isotope Ratio Mass Spectrometer (EA-CF/IRMS; PDZ Europa GSL / 20-20) (Section 3.2.8). Samples from plots that did not receive $^{15}$N amended urine were riffling and ground first to minimise the risk of cross contamination of $^{15}$N. All soil grinding equipment was thoroughly cleaned between treatments. The $^{15}$N recovered from the total soil N, as a percentage of the total $^{15}$N applied as urine, was then calculated using Equation 5.1.

5.2.6 Data and statistical analysis

Unless specified differently, all statistical calculations were performed using the ANOVA directive of GenStat (15th Edition). The “least significant difference” (LSD) between the sample means at the 5% significance level is presented in most graphical figures and tables. The “standard error of the difference between sample means” (SED) can be calculated by dividing the LSD (5%) by the t value (at the 5% significance level). For example, in the current study, when comparing between plots there was 6 degrees of freedom, therefore $t = 2.45$, and the SED (5%) was equal to the LSD (5%) divided by 2.45. When comparing between zones there was 24 degrees of freedom, therefore $t = 2.06$, and the SED (5%) was equal to the LSD divided by 2.06.

When comparing treatment effects on pasture yield, N content and N uptake between entire plots, a weighted mean was calculated (shown by Equation 5.7 below), using pasture yield as an example) to account for the differences in surface area between Zones A, B and C.
Weighted Mean

\[
\frac{(Yield^a \times Area^a) + (Yield^b \times Area^b) + (Yield^c \times Area^c)}{Area^{a+b+c}}
\]

Where \(Yield^a\), \(Yield^b\) and \(Yield^c\) = the measured yields from Zones A, B and C; \(Area^a\), \(Area^b\) and \(Area^c\) = the surface areas (m\(^2\)) of Zones A, B and C; and \(Area^{a+b+c}\) = the area of the total plot.
5.3 Results

5.3.1 Climate data

Total rainfall over the duration of the experiment was 1088 mm (Figure 5.3). From the start of the experiment (17 Oct 2011) through to the end of November there was 66 mm, less rainfall than average. Then in December 2011 and January 2012 there was 197 and 96 mm of rainfall, respectively, which far exceeded the average summer rainfall.

Average air temperature over the experimental period was 13.9°C and the average soil temperature (10 cm depth) was 14.8°C (Figure 5.4). There was a total of 15 frost days which occurred between 23 May and 19 Jul 2012.

Figure 5.3 Daily (left axis) and cumulative (right axis) rainfall at the field site, from 1 October 2011 to 1 August 2012.
5.3.2 Pasture

A total of 8 pasture harvests were carried out on the following dates: 31 Oct 2011 (day 14); 18 Nov 2011 (day 32); 14 Dec 2011 (day 58); 10 Jan 2012 (day 85); 31 Jan 2012 (day 106); 27 Feb 2012 (day 133); 30 Mar 2012 (day 165); and 7 May 2012 (day 203). Two more pasture harvests were carried out on 12 Jun 2012 (day 239) and 23 Aug 2012 (day 311) for pasture $^{15}$N analysis only.

5.3.2.1 Temporal pasture yield

From the first harvest (31 Oct 2011) through to February 2012 there were visually recognisable increases in pasture growth in the plots that received urine (see Figure 5.5a and Figure 5.5b). There were no visual differences between the fertiliser and non-fertiliser affected plots. The effect of fertiliser addition on pasture yield was not significant on any individual harvest, nor cumulatively by plot, or by zone. There was no interaction between the fertiliser and urine treatments on pasture yield, at any time, by plot or by zone.
Plot scale

At the whole plot scale (Zones A+B+C), pasture yields generally decreased over the 200 time period in all treatments, with the exception of a considerable increase in DM yield on day 85, followed by a decrease the following harvest (day 106) (Figure 5.6). Pasture DM yields ranged from 720-1700; 530-1500; 530-1300 and 480-1300 kg DM ha\(^{-1}\) for the urine + fertiliser, urine only, fertiliser only and control treatments, respectively. DM yields were greater in the urine treatments than the non-urine treatment at each harvest until day 85, however, these differences were only significant (p < 0.001) on the second and third harvests (days 32 and 58).

Figure 5.6 Mean (weighted) pasture yields from the whole plot area, from days 0 to 239. Error bars = LSD (5%), n = 4.
**Zone Scale**

Pasture DM yields in the individual zones decreased with time in the urine treatments, with the exception of the increase on day 85 in Zones B and C, and subsequent decrease on day 106. In the non-urine treatments there was a similar overall pattern with time, however, on day 58 there was a considerable increase in DM yield in Zone A only (p < 0.001), while Zones B and C decreased (Figure 5.7c & Figure 5.7d). Mean DM yields from Zone A ranged from 900-2200; 700-2150; 530-2000 and 570-1300 kg DM ha⁻¹; yields in Zone B ranged from 800-1800; 500-1600; 500-1400 and 550-1400 kg DM ha⁻¹; and yields in Zone C ranged from 450-1500; 500-1400; 180-1200 and 200-1200 kg DM ha⁻¹ in the urine + fertiliser, urine only, fertiliser only, and control treatments, respectively.

In the urine treatments, DM yields from Zone A were > Zone B and C (p < 0.001) in the first 3 harvests, up to day 58; and were > Zone C (p < 0.05) on day 85. Pasture DM yields from Zone B were > Zone C (p < 0.05) on days 32, 58 and 239 in the case of urine + fertiliser, and days 14 and 32 in the case of the urine only treatments (Figure 5.7a & b).

The DM yields in Zone A of the non-urine treatments were > Zones B and C (p< 0.001) on day 58 (Figure 5.7c & Figure 5.7d). Dry matter yield did not differ between Zones A, B and C at any other time during the experiment.
5.3.2.2 Cumulative pasture yield

Plot scale

At the whole plot scale, total cumulative pasture DM yield ranged from 7000-9000 kg DM ha\(^{-1}\), and was highest in the presence of urine (p < 0.05) (Figure 5.8). There was an apparent fertiliser effect within the urine affected treatments, where the total cumulative DM yield from the urine + fertiliser and urine only treatments was 9000 and 7800 kg DM ha\(^{-1}\), respectively; a difference of 1200 kg DM ha\(^{-1}\); however, this was not significant at the 5% level (Figure 5.10a). The cumulative DM yield from the urine + fertiliser treatment was greater (p < 0.05) than that from both non-urine treatments, however, the cumulative DM yield from the urine only treatment was not.
Zone Scale

Cumulative DM yields at the zone scale ranged from 7500-13000; 6300-8300 and 5600-6700 in Zones A, B and C, respectively (Figure 5.9 and Figure 5.10b). In the urine affected treatments, cumulative DM yields from Zone A were greater (p < 0.001) than those from Zone B and Zone C. Cumulative DM yields from Zone B were not significantly different from Zone C. In the non-urine affected treatments, there was no difference in cumulative DM yield between Zones A, B or C.

Figure 5.8 Mean (weighted) cumulative pasture yields from the whole plot scale, from days 0 to 239. Error bars = LSD (5%), n = 4.
Figure 5.9 Mean cumulative pasture yields from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.
5.3.2.3 Pasture N content

Plot scale

The mean weighted pasture N content at the whole plot scale ranged from 2.1-3.0; 2.2-3.1; 2.2-3.2 and 2.5-3.1 % N in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively, over the duration of the experiment.

Pasture N content in the non-urine treatments increased slightly, but, generally, did not change over time in the urine treatments. The exception was a decline of between 0.3-0.6% N on day 133 in all treatments.

Figure 5.10 Cumulative pasture yield (a) at the plot scale (weighted means) and (b) from Zones A, B and C for all treatments. Different letters indicate a significant difference. Error = LSD (5%), n = 4.
Urine caused pasture N content to be greater (p < 0.001) than in the non-urine treatments at the first two harvests (days 14 and 32). By day 58, N content in the urine + fertiliser treatment remained higher than that in the non-urine treatments (p < 0.05) but the urine only treatment did not (Figure 5.11).

The effect of fertiliser addition on pasture N content was not significant at the plot scale, on any harvest. There was no treatment interaction due to urine and fertiliser on pasture N content at any time.

![Figure 5.11 Mean (weighted) pasture N content from whole plot area from days 0 to 239. Error bars = LSD (5%), n = 4.](image)

**Zone scale**

At the zone scale, mean pasture N contents from Zone A ranged from 2.7-4.4; 2.4-4.3; 2.3-3.0 and 1.9-2.8 % N; from Zone B, yields ranged from 2.5-3.3; 2.3-3.4; 2.2-2.9 and 2.1-2.8 % N; and from Zone C, yields ranged from 2.2-3.1; 2.1-3.0; 2.1-3.0 and 2.1-2.9 % N in the urine + fertiliser, urine only, fertiliser only, and control treatments, respectively (Figure 5.12). There was a urine effect where the pasture N contents of Zones A and B in the urine treatments were greater (p < 0.05) than those of all zones in the non-urine treatments from day 14-58. Pasture N content in Zone C did not differ between treatments.

The pasture N content in the urine treatments was greater (p < 0.001) in Zone A, compared to Zone B and C on days 14 and 32. After this, there was no difference
between zones, with the exception of day 58 in the urine only treatment (p < 0.05) (Figure 5.12a and Figure 5.12b). In the non-urine treatments, there was no fertiliser effect or difference in N content between Zones A, B or C at any time during the experiment (Figure 5.12c and Figure 5.12d).

![Figure 5.12](image-url)  Mean N content from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.

### 5.3.2.4 Temporal pasture N uptake

**Plot scale**

In the urine treatments, N uptake decreased over time, with the exception of day 85, where increases of 24 and 18 kg N ha\(^{-1}\) occurred in the urine + fertiliser and urine only treatments, respectively, followed by a reduction of 25 and 21 kg N ha\(^{-1}\), respectively, at the following harvest on day 106 (Figure 5.13). Pasture N uptake in the non-urine treatments initially decreased over time (Figure 5.13) before a similar increase in N
uptake occurred on day 85, as described above, with a smaller reduction on day 106 of about 14 kg N ha\(^{-1}\).

Pasture N uptake was greater (p < 0.001) in urine treatments than in non-urine treatments on the first two harvests (days 14 and 32). By days 58 and 85, N uptake in the urine + fertiliser treatment was higher (p < 0.05) than both non-urine treatments, but N uptake in the urine only treatment was not. After day 85, although N uptake in the urine + fertiliser treatment appeared to be consistently greater than in other treatments (Figure 5.13), there was no statistical difference between any of the treatments at individual harvests.

The effect of fertiliser addition on pasture N uptake was not significant at the plot scale on any individual harvest. There was also no interaction between the urine and fertiliser treatments on pasture N content at any time.

![Figure 5.13](image)

**Figure 5.13** Mean (weighted) pasture N uptake from whole plot area (mean of Zones A, B and C) from days 0 to 239. Error bars = LSD, n = 4.

*Zone scale*

Pasture N uptake in Zones A and B in the urine-affected treatments generally decreased with time, but not in Zone C. On day 85, N uptake occurred in Zones B and C, but not Zone A, and the subsequent decrease on day 106 occurred in all zones (Figure 5.14a and Figure 5.14b). In the non-urine treatments there was generally no change in N uptake over time, however, on day 58, there was an increase in N uptake in Zone A (p < 0.001), while Zones B and C decreased. Similar to the urine-affected treatments, pasture N
uptake increased in Zones B and C on day 85, but not Zone A, which was followed by a decrease in N uptake across all zones on day 106. Mean pasture N uptake from Zone A ranged from 31-95; 22-90; 18-50 and 15-34 kg N ha\(^{-1}\); N uptake from Zone B ranged from 27-55; 19-42; 13-41 and 15-34 kg N ha\(^{-1}\); and N uptake from Zone C ranged from 13-45; 14-39; 5-34 and 5-36 kg N ha\(^{-1}\) in the urine + fertiliser, urine only, fertiliser only, and control treatments, respectively.

There was a urine effect where pasture N uptake in Zone A of the urine treatments, was greater (p < 0.001) than in Zone B and C in the first 3 harvests, up to 58 days post treatment application, and N uptake in Zone A of the urine + fertiliser treatment was significantly higher (p < 0.05), than Zone C on day 85, but not Zone B. Pasture N uptake from Zone B was greater (p < 0.001) than Zone C on days 14, 32 and 58 (first 3 harvests) and days 14 and 32 (first 2 harvests) from the urine + fertiliser and urine only treatments, respectively (Figure 5.14a and Figure 5.14b). In the non-urine affected treatments, N uptake from Zone A was higher than from Zones B and C (p< 0.001) on day 58. There were no other statistical differences between zones in the non-urine affected treatments (Figure 5.14c and Figure 5.14d). Fertiliser had no effect on N uptake at the zone scale, neither was there any interaction between the urine and fertiliser treatments.
5.3.2.5 Cumulative pasture N uptake

Plot scale

At the plot scale, total cumulative N uptake was highest in the presence of urine with 260, 218, 185 and 182 kg N ha\(^{-1}\) in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively (Figure 5.15 and Figure 5.17a). Total cumulative N uptake from the urine + fertiliser treatment was greater (\(p < 0.05\)) than from the non-urine treatments, however, the total cumulative N uptake from the urine only treatment was not.

---

Figure 5.14 Mean pasture N uptake from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), \(n = 4\).
At the zone scale, mean total cumulative N uptake ranged from 190-455, 180-275 and 175-200 kg N ha\(^{-1}\) in Zones A, B and C, respectively, across all treatments (Figure 5.16 and Figure 5.17b). In the urine-affected treatments, cumulative N uptake from Zone A was greater than those from Zone B and Zone C (p < 0.001). However, total cumulative N uptake from Zone B was not different from Zone C. The difference in total cumulative N uptake in Zone A between the urine + fertiliser and urine only treatments was 71 kg N (p > 0.05), yet the difference between total cumulative N uptake in Zone A of the fertiliser only and control treatments was only 35 kg N (p > 0.05). In both cases (urine and non-urine) the only point of difference was an application of 35 kg N ha\(^{-1}\) fertiliser, and although there was no statistical difference between total cumulative N uptake in the urine + fertiliser and urine only, or fertiliser only and control treatments, the apparent fertiliser associated total N uptake in Zone A under urine was still double that under no urine (Figure 5.17b).
Figure 5.16 Mean cumulative pasture N uptake from Zones A, B and C for treatments (a) urine + fertiliser, (b) urine only, (c) fertiliser only and (d) control from days 0 to 239. Error bars = LSD (5%), n = 4.
Figure 5.17 Mean cumulative N uptake (a) from the plot scale and (b) from Zones A, B and C for all treatments. Error = LSD (5%), n = 4.

5.3.2.6 Temporal pasture $^{15}$N recovery

Recovery of $^{15}$N in the pasture was greatest at the beginning of the experiment in both treatments that received urinary $^{15}$N, and declined with time (Figure 5.18). In the case of the urine + fertiliser treatment, $^{15}$N recovery was highest (8%) at the first harvest, then declined rapidly until day 106, after which, it continued to decline at a much slower rate. In the urine only treatment, $^{15}$N recovery increased in Zone A and B on the second harvest (day 32) before declining in the same manner as the urine + fertiliser treatment. By day 106, pasture $^{15}$N recovery, at harvest, in both treatments was < 1.5% and by day 311 was 0.35%.
In both treatments, pasture $^{15}$N recovery from Zone A was greater ($p < 0.001$) than that from Zones B and C at every harvest. Pasture $^{15}$N recovery from Zone B was greater ($p < 0.001$) than Zone C at every harvest, with the exception of day 203 in the urine only treatment (Figure 5.18). Pasture $^{15}$N recovery in Zone C was above natural abundance levels until day 106. The effect of fertiliser addition on $^{15}$N recovery was not significant by zone at any individual harvest. Within zones, there was no interaction between the urine and fertiliser treatments on pasture $^{15}$N recovery at any time.

![Figure 5.18](image_url)

**Figure 5.18** Pasture $^{15}$N recovery over time from Zones A, B and C for treatments (a) urine + fertiliser and (b) urine only from day 0 to 310. Error = LSD (5%), $n = 4$.

### 5.3.2.7 Cumulative pasture $^{15}$N recovery

Cumulative pasture $^{15}$N recovery increased most rapidly from day 14 to day 106, reaching 27, 15 and 3.5% recovery in Zones A, B and C, respectively from the urine + fertiliser treatment, and 27, 15 and 2% recovery in Zones A, B and C of the urine only treatment. Increases after this time were insignificant (Figure 5.19).
Total cumulative pasture $^{15}$N recoveries were not significantly different between the urine + fertiliser and urine only treatments, nor were the individual pasture $^{15}$N recoveries from Zones A, B and C significant (Figure 5.20). As such, there was no effect of fertiliser addition on cumulative $^{15}$N recovery at any time during the experiment. There was also no interaction between the urine and fertiliser treatments on cumulative $^{15}$N recovery.

Within each individual treatment, the cumulative $^{15}$N recovery from Zone A was significantly greater than from Zones B and C at every harvest, and cumulative recovery from Zone B was greater than Zone C at every harvest (Figure 5.19).

**Figure 5.19** Cumulative pasture $^{15}$N recovery from Zones A, B and C for treatments (a) urine + fertiliser and (b) urine only, from day 0 to 310. Error = LSD (5%), $n = 4$
Figure 5.20  Mean total cumulative pasture $^{15}$N recovery (%) from Zones A, B and C for urine + fertiliser and urine only treatments.  Error = LSD (5%), n = 4.

The total cumulative pasture $^{15}$N uptake and background non-$^{15}$N uptake were calculated on a per m$^2$ basis and are shown by Figure 5.21.  The N uptake was greatest from the background soil N pool than from the labelled urinary $^{15}$N pool ($p < 0.001$).  There was no statistical difference in total pasture $^{15}$N uptake or background N uptake between the two treatments.  In both the U+F+ and U+F0 treatments, the cumulative pasture $^{15}$N and background N uptake on a per m$^2$ basis was significantly greater in Zone A compared to Zones B and C ($p < 0.001$), and in Zone B compared to Zone C ($p < 0.001$) (Figure 5.21).  In other words, from the edge of the wetted area, the urinary and background N uptake by pasture declined significantly with increasing distance from the wetted area.  The total N uptake is not shown by Figure 5.21, but can be calculated as the sum of corresponding points in Figure 5.21a and Figure 5.21b.  The plant $^{15}$N uptake as a percentage of the total N uptake was 30, 16 and 10% in the U+F+ treatment, and 29, 15, and 3% in the U+F0 treatment, for Zones A, B and C respectively (Figure 5.21a).  The background, non-labelled plant N uptake as a percentage of the total N uptake was 70, 84 and 90% in the U+F+ treatment, and 71, 85, and 97% in the U+F0 treatment, for Zones A, B and C, respectively (Figure 5.21b).
Pasture cuttings, $^{15}$N atom %

Pasture uptake of $^{15}$N in the urine-affected plots increased considerably in the first 10 days following treatment application in all zones. There was a consistent and significant zone effect on all days ($p < 0.001$) with Zone A having the highest $^{15}$N enrichment followed by Zone B, then C. The urinary $^{15}$N uptake was slightly but consistently lower in the presence of fertiliser ($p < 0.05$). From days 10 to 58, the $^{15}$N atom % levels plateaued (or decreased slightly in the case of Zone B) and following this, they steadily decreased for the remainder of the experiment (Figure 5.22). By day 311, the pasture was still $^{15}$N enriched in all zones of both treatments compared to control levels (mean of 0.3709%).

Figure 5.21 Total cumulative pasture (a) $^{15}$N uptake and (b) background soil N uptake with distance from the centre of the urine patch in Zones A, B and C. Note the y axes differ.
5.3.3 Soil N

5.3.3.1 Gravimetric soil moisture content

Soil moisture content was consistently greater \((p < 0.05)\) in the surface depth (0-7.5 cm) compared to the deeper depth (7.5-20 cm) until day 21. In the surface depth, average soil moistures ranged from 62-35, 62-35, 63-38 and 64-36% in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively. In the second depth, average soil moistures ranged from 54-38, 53-37, 52-41 and 55-39% in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively. Soil moisture remained fairly constant with time in both depths from day 1 to day 21 (between 50 and 65%). However, on day 43, soil moistures from both depths declined to between 33 and 38%, then by day 63 they had increased again, but this was followed by another large decline on day 106.
In the urine-affected treatments, soil moisture in the surface depth of Zone C was significantly lower (p < 0.05) than those in Zones A and B on day 63. This was followed by a large increase in soil moisture in Zone C at day 106, whereas all other samples decreased on this day. This did not occur in the non-urine treatments. There were no other significant differences in the soil moisture content between the different zones within any given treatment, nor across treatments. Furthermore, there was no fertiliser effect, nor any interaction between the urine and fertiliser treatments on soil moisture.

![Graph showing soil moisture content at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4.]

**Figure 5.23** Soil moisture content at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4.

### 5.3.3.2 Soil NH₄⁺-N

**Plot scale**

In the 0-7.5 cm depth, soil NH₄⁺-N concentrations ranged from 0.3-62 mg kg⁻¹ soil. Soil NH₄⁺-N concentrations were greater (p < 0.001) in the urine-affected treatments from day 1-14. Soil NH₄⁺-N concentrations peaked on day 1 at 62 and 58 mg NH₄⁺-N kg⁻¹ soil in
the urine + fertiliser and urine only treatments, respectively. By day 21, these concentrations had returned to control levels of between 2 and 7 mg NH$_4^+$-N kg$^{-1}$ soil (Figure 5.24a). There were only small peaks in soil NH$_4^+$-N concentrations in the non-urine treatments, with a slight increase on day 14 of 3 and 6 mg NH$_4^+$-N g$^{-1}$ soil in the fertiliser only and control treatments, respectively. There was no consistent effect of fertiliser on the urine treatments.

In the 7.5-20 cm depth, soil NH$_4^+$-N concentrations ranged from 0.2-9.5 mg kg$^{-1}$ soil. Soil NH$_4^+$-N concentrations in the urine treatments were greater (p < 0.05) than in the non-urine treatments on days 8, 14 and 21 (Figure 5.24b). There was no fertiliser effect on soil NH$_4^+$-N concentrations at this depth. There was no interaction between urine and fertiliser on soil NH$_4^+$-N concentrations at the plot scale.

Zone scale

In the 0-7.5 cm depth, soil NH$_4^+$-N concentrations peaked on day 1 at 726, 580 and 47 mg NH$_4^+$-N kg$^{-1}$ soil in the urine + fertiliser, urine only and fertiliser only treatments, respectively. In the 7.5-20 cm depth, soil NH$_4^+$-N concentration peaks were lower at 93 and 236 mg NH$_4^+$-N kg$^{-1}$ soil in the urine + fertiliser, and fertiliser only treatments, respectively. Negligible peaks occurred in the control at both depths. At both depths, soil

Figure 5.24  Soil NH$_4^+$-N concentrations at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth under urine + fertiliser (U+F+), urine only (U+F0), fertiliser only (U0F+) and the control (U0F0) from day 0 to 106. Error bars = LSD (5%), n = 4. Note the y axes differ.
NH$_4^+$-N concentrations were greater (p < 0.001) in the urine-affected treatments than the non-urine treatments from day 1-21. In the non-urine treatments, soil NH$_4^+$-N concentrations were greater (p < 0.05) in the fertiliser only treatment on day 1 only. There was no significant fertiliser effect, nor any interaction between the urine and fertiliser treatments on soil NH$_4^+$-N on any sampling day.

In the 0-7.5 cm depth, soil NH$_4^+$-N concentrations under the urine treatments were greater (p < 0.001) in Zone A compared to Zones B and C, from day 1-21; and greater in Zone B compared to Zone C from day 1-14 (Figure 5.25a & b). After this, soil concentrations equalled control levels with no difference between zones. In the non-urine treatments, soil NH$_4^+$-N concentrations were greater (p < 0.05) in Zone A compared to Zones B and C, on day 1 only (Figure 5.25c).

In the 7.5-20 cm depth, soil NH$_4^+$-N concentrations in the urine treatments were greater (p < 0.001) in Zone A compared to Zones B and C, from day 1-21; and greater in Zone B compared to Zone C on day 1 only (Figure 5.25a & Figure 5.25b). In the non-urine treatments soil NH$_4^+$-N concentrations were greater (p < 0.05) in Zone A compared to Zones B and C, on day 1 only (Figure 5.25c). There was no difference in soil NH$_4^+$-N concentrations between zones in the control at either depth (Figure 5.25d). There was no interaction between the urine and fertiliser treatments on soil NH$_4^+$-N concentrations at the zone scale.
5.3.3.3 Soil NO$_3^-$-N

**Plot scale**

In the 0-7.5 cm depth, mean soil NO$_3^-$-N concentrations ranged from 3-41 mg kg$^{-1}$ soil. Soil NO$_3^-$-N was greater ($p < 0.001$) in the urine-affected treatments from day 8 to day 63 and soil NO$_3^-$-N peaked at 36 and 41 mg kg$^{-1}$ soil in the urine + fertiliser and urine only treatments, respectively (Figure 5.26a). In the 7.5-20 cm depth, mean soil NO$_3^-$-N concentrations peaked from 1-12 mg kg$^{-1}$ soil, which occurred in the urine + fertiliser and urine only treatments on day 21. Soil NO$_3^-$-N concentrations were greater ($p < 0.001$) in the urine-affected treatments from day 8-63 (Figure 5.26b). There was no fertiliser effect or interaction between the urine and fertiliser treatments on soil NO$_3^-$-N concentrations at either depth at any time.
Zone scale

In the 0-7.5 cm depth, soil NO$_3$-N concentrations peaked in Zone A on day 21 at 427, and 307 mg NO$_3$-N g$^{-1}$ soil in the urine + fertiliser and urine only treatments, respectively, and at 15.2 mg NO$_3$-N g$^{-1}$ soil on day 1 in the fertiliser only treatment. In Zone A in the 7.5-20 cm depth, soil NO$_3$-N concentration peaks were much lower, at 207 and 174 mg NO$_3$-N g$^{-1}$ soil on day 21 in the urine + fertiliser, and urine only treatments, respectively. No peaks occurred in the fertiliser only treatment at this depth, and no peaks occurred in the control at either depth. Only on day 1 were soil NO$_3$-N concentrations in the urine treatments greater ($p < 0.001$) in Zone B than Zone A and C. Otherwise, soil NO$_3$-N concentrations were greater ($p < 0.001$) in Zone A compared to Zones B and C from day 8-63; and were greater ($p < 0.05$) in Zone B compared to Zone C from day 8-41 (Figure 5.27a and Figure 5.27b). In the non-urine treatments, the soil NO$_3$-N concentrations were greater ($p < 0.05$) in Zone A compared to Zones B and C, on day 1 only (Figure 5.27c) and there was no difference in soil NO$_3$-N concentrations between zones in the control (Figure 5.27d).

In the 7.5-20 cm depth, soil NO$_3$-N concentrations under the urine affected treatments were greater ($p < 0.001$) in Zone A compared to Zones B and C, from day 8-63; and in
Zone B compared to Zone C from day 8-21 (Figure 5.27a & b). In the fertiliser only treatment, the soil NO$_3^-$-N concentration was greater (p < 0.05) in Zone A compared to Zones B and C, on day 1 only (Figure 5.25c). There was no difference in soil NO$_3^-$-N concentrations between zones in the control (Figure 5.27d).

At both depths, soil NO$_3^-$-N concentrations were greater (p < 0.001) in the urine treatments than the non-urine treatments from day 8-63. There was no significant fertiliser effect, nor any urine and fertiliser interaction on soil NO$_3^-$-N at either depth on any sampling occasion.

**Figure 5.27** Soil NO$_3^-$-N concentration at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ.
5.3.3.4 Soil N content (%)

Plot scale

Soil N content ranged from 0.66-0.81% in the 0-7.5 cm depth and from 0.50-0.59 % in the 7.5-20 cm depth. At the plot scale, there was no significant difference in the soil N content between any of the four treatments at either the 0-7.5 or 7.5-20 cm depth, and there was no significant change in the soil N content with time during the experiment (Figure 5.28). There was also no interaction between the urine and fertiliser treatments on the soil N content.

![Figure 5.28 Soil N content (%) at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth for treatments (U+F+) urine + fertiliser; (U+F0) urine only; (U0F+) fertiliser only and (U0F0) control, from day 0 to 106. Error = LSD (5%), n = 4.](image)

Zone scale

Mean soil N content at the zone scale ranged from 0.65-0.88, 0.61-0.88, 0.66-0.78 and 0.66-0.87% in the 0-7.5 cm depth and 0.48-0.58, 0.48-0.63, 0.52-0.59 and 0.52-0.61% in the 7.5-20 cm depth from the urine + fertiliser, urine only, fertiliser only and control treatments, respectively, (Figure 5.29).

In the urine treatments, at the 0-7.5 cm depth, the soil N content was greater (p < 0.05) in Zone A, followed by Zone B, then Zone C from day 1-43. After this time, there was no difference between zones. In the case of the urine only treatment, the mean soil N content of Zone A declined to be below that of Zone B on day 43, and then declined further to be below both Zones B and C on day 63. At the 7.5-20 cm depth, although the soil N
content in Zone A tended to be slightly greater than in the other zones, there was no statistical difference between them (Figure 5.29a and Figure 5.29b).

In the non-urine treatments, there was no difference in soil N content between Zones at either depth, with the exception of one instance on day 21 in the control treatment, where the soil N content in Zone B at the 0-7.5 cm depth was greater (p < 0.05) than that in Zones A and C (Figure 5.29c and Figure 5.29d). There was no fertiliser effect on soil N and no interaction between the urine and fertiliser treatments on the soil N content at any time at either depth.

![Figure 5.29 Soil N content (%) at two depths (0-7.5 and 7.5-20 cm) from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control from day 0 to 106. Error = LSD (5%), n = 4.](image)

5.3.3.5 Total soil N (kg N ha⁻¹)

**Plot scale**

The total soil N (in kg N ha⁻¹) at the plot scale shown by Figure 5.30 is the sum of the soil N (kg N ha⁻¹) from within the 0-7.5 and 7.5-20 cm depths. It is presented like this
because the volume of soil in each of the two depth profiles is different, and would result in an invalid comparison of the mass of N ha$^{-1}$ of a 7.5 cm depth profile with a 12.5 cm depth profile. Total soil N at the plot scale ranged from 9660-11400 kg N ha$^{-1}$. There was no significant urine or fertiliser effect on the total soil N in any of the four treatments, and there was no change in the total soil N with time. There was also no interaction between the urine and fertiliser treatments on the total soil N.

Figure 5.30  Soil total N (kg N ha$^{-1}$) at the plot scale from 0-20 cm depth (sum of 0-7.5 and 7.5-20 cm depths) from (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control treatment from day 0 to 106. Error = LSD (5%), n = 4.

Zone scale

Total soil N from 0-20 cm (sum of 0-7.5 cm and 7.5-20 cm depths) at the zone scale is presented in Figure 5.31. Mean total N ranged from 9600-11800, 9100-11800, 9900-11200 and 9900-11800 kg N ha$^{-1}$ in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively.

In both urine treatments, total soil in Zones A and B increased until day 14 decreased after that. There was a urine effect by zone (p < 0.05) from day 1-43 in the urine + fertiliser treatment, and from day 1-21 in the urine only treatment, where the total soil N in Zone A was greater than in Zones B and C. In the case of the urine + fertiliser treatment, after day 43, there was no difference in total soil N between all three zones (Figure 5.31a and Figure 5.31b). There was no difference in total soil N between zones in
the non-urine treatments. There was no fertiliser effect on any treatments (Figure 5.31c and Figure 5.31d). There was also no interaction between the urine and fertiliser treatments on the total soil N.

**Figure 5.31 Soil total N (kg N ha\(^{-1}\)) from Zones A, B and C from 0 - 20 cm depth (sum of 0-7.5 and 7.5-20 cm depths) from (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control treatment from day 0 to 106. Error = LSD (5%), n = 4.**

### 5.3.3.6 Soil inorganic \(^{15}\)N Recovery

Mean soil inorganic \(^{15}\)N recovery (NH\(_4^+\)\(^{15}\)N + NO\(_3^-\)\(^{15}\)N) decreased with time in both depths. Most of the inorganic \(^{15}\)N recovered was from the NO\(_3^-\)-N pool. In Zone C, of both urine treatments, and at both depths, the concentration of inorganic N in the soil extracts was too low to facilitate \(^{15}\)N diffusion analysis, so \(^{15}\)N recovery in Zone C was negligible and given values of zero.

*Plot scale*

In the 0-7.5 cm depth, soil inorganic \(^{15}\)N recovery from the whole plot ranged from 66 and 82% at day 1, to 13.0 and 8.3% at day 43 in the urine + fertiliser and urine only
treatments, respectively (Figure 5.32a and Figure 5.32c). In the 7.5-20 cm depth, inorganic soil $^{15}$N recovery ranged from 30 and 6% on day 1, to 10 and 3% on day 106, in the urine + fertiliser and urine only treatments, respectively (Figure 5.32b and Figure 5.32d). Total $^{15}$N recovered at the end of the experiment is shown in Figure 5.34 which differentiates the $^{15}$N recovered in the inorganic and organic N pools at each depth.

**Zone scale**

Soil inorganic $^{15}$N recovery in the 0-7.5 cm depth ranged from 13-43% and 8-44% in Zone A for the urine + fertiliser and urine only treatments, respectively, (Figure 5.32); while the corresponding values for Zone B were 0-25% and 0-38%, respectively. Under both treatments, $^{15}$N recovery as inorganic N was greatest from Zone A, followed by Zone B, where $^{15}$N was recovered only on days 1 and 8, followed by Zone C, with zero recovery. However, there was large variation in the data. Inorganic $^{15}$N recovery from Zone A was greater than zero ($p < 0.05$) on all days except day 1, but not greater than Zone B in the urine + fertiliser and urine only treatments. Inorganic $^{15}$N recovery from Zone B was not statistically greater that zero in either treatment on day 1 or 8 (Figure 5.32a and Figure 5.32b).

In the 7.5-20 cm depth, soil inorganic $^{15}$N recovery from Zone A ranged from 31-21% in the urine + fertiliser and urine only treatments, respectively. There was zero recovery from Zones B and C. In the urine + fertiliser treatment, inorganic $^{15}$N recovery was greater ($p < 0.05$) than zero from day 14 onwards (Figure 5.32c), but in the urine only treatment, inorganic $^{15}$N recovery was not statistically greater than zero on any sampling day (Figure 5.32d).

There was no effect of fertiliser and no interaction between the urine and fertiliser treatments on soil inorganic $^{15}$N recovery on any sampling day at either depth.
Figure 5.32  Soil inorganic $^{15}$N recovery (NH$_4^+$-$^{15}$N + NO$_3^-$-$^{15}$N) from day 0 to 43 from the urine + fertiliser treatment at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth and from the urine only treatment at (c) 0-7.5 cm depth and (d) 7.5-20 cm depth. Error bars = LSD (5%), n = 4.

5.3.3.7 Soil organic $^{15}$N recovery

Plot scale

Soil organic $^{15}$N recovery was calculated by subtracting the inorganic $^{15}$N recovered from the total $^{15}$N recovered. Soil organic $^{15}$N recovery in the 0-7.5 cm depth at the whole plot scale ranged from 8.8-24% and 6.8-31% in the urine + fertiliser and urine only treatments, respectively (Figure 5.33a and Figure 5.33c). In the 7.5-20 cm depth, soil organic $^{15}$N recovery (sum of Zones A, B and C) ranged from 4.7-10% and 3.8-15% in the urine + fertiliser and urine only treatments, respectively (Figure 5.33b and Figure 5.33d). The soil organic $^{15}$N recovered at each depth at the end of the experiment is shown in Figure 5.34. There was no fertiliser effect or interaction between the urine and fertiliser treatments on soil organic $^{15}$N recovery at the plot scale at either depth.
Zone scale

In the 0-7.5 cm depth, soil organic $^{15}$N recovery in Zone A ranged from 8 (day 1) to 12% (day 106), and 9 (day 1) to 13.5% (day 106) in the urine + fertiliser and urine only treatments, respectively. In Zone B soil organic $^{15}$N recovery ranged from 12 (day 1) to 3% (day 106), and from 8 (day 1) to 7% (day 106) in the urine + fertiliser and urine only treatments, respectively. Soil organic $^{15}$N recovery in the urine + fertiliser treatment was greater ($p < 0.001$) in Zone A compared to Zones B and C on all days except 1, 8 and 43; and was greater ($p < 0.05$) in Zone B compared to Zone C on day 1 only (Figure 5.33a).

In the urine only treatment, soil total $^{15}$N recovery was greater ($p < 0.001$) in Zone A compared to Zones B and C on days 21, 63 and 106; and was greater in Zone B than Zone C on days 8 and 14. Soil organic $^{15}$N recovery in Zones A and B of both treatments declined substantially on day 43, before recovering over the ensuing dates (Figure 5.33a and Figure 5.33c).

In the 7.5-20 cm depth, soil organic $^{15}$N recovery in Zone A ranged from 6 (day 1) to 3.2% (day 106), and 1.5 (day 1) to 5.3% (day 106) in the urine + fertiliser and urine only treatments, respectively. In Zone B soil organic $^{15}$N recovery ranged from 2.5 (day 1) to 0.5% (day 106), and from 1.5 (day 1) to 3% (day 106) in the urine + fertiliser and urine only treatments, respectively. Soil organic $^{15}$N recovery in the urine + fertiliser treatment was greater ($p < 0.05$) in Zone A compared to Zones B and C on days 14 and 21; with no difference between Zone B and Zone C on any sampling day (Figure 5.33b). In the urine only treatment, soil organic $^{15}$N recovery was greater ($p < 0.05$) in Zone A compared to Zones B and C on day 21 only; and was greater in Zone B than Zone C on day 8 only.

On day 63, soil organic $^{15}$N recovery in Zone C increased substantially; however, this was again due to a single outlier in one of the four replicates, and not statistically significant (Figure 5.33d).

There was a fertiliser effect ($p < 0.05$) on day 8 where soil organic $^{15}$N recovery from Zone B in the urine only treatment was greater than in the urine + fertiliser treatment.

There was also a fertiliser effect ($p < 0.05$) on day 21 where soil organic $^{15}$N recovery from Zone A in the urine only treatment was greater than in the urine + fertiliser treatment.

There was no interaction between the urine and fertiliser treatments on soil organic $^{15}$N recovery on any sampling day at either depth.
Figure 5.33  Soil organic $^{15}$N recovery from day 0 to 43 from the urine + fertiliser treatment at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth and from the urine only treatment at (c) 0-7.5 cm depth and (d) 7.5-20 cm depth. Error bars = LSD (5%), n = 4. Organic $^{15}$N recovery calculated as the difference of total and inorganic $^{15}$N recovery.

Figure 5.34  Soil $^{15}$N recovered from the inorganic and organic fractions at the end of the trial at (a) 0-7.5 cm and (b) 7.5-20 cm depths. Error = LSD (5%), n = 4.
5.3.3.8 Soil total $^{15}$N recovery

Plot scale

Mean total soil $^{15}$N recovery decreased with time in both treatments and at both depths. In the 0-7.5 cm depth, total soil $^{15}$N recovery from the whole plot ranged from 88 and 99.8% on day 1, to 13.5 and 11.4% on day 106 in the urine + fertiliser and urine only treatments, respectively, (Figure 5.35a and Figure 5.35c). In the 7.5-20 cm depth, total $^{15}$N recovery ranged from 41 and 44% on day 1, to 4.7 and 7.1% on day 106, in the urine + fertiliser and urine only treatments, respectively (Figure 5.35b and Figure 5.35d).

Zone scale

In the 0-7.5 cm depth, total $^{15}$N recovery in Zone A ranged from 52 and 52% on day 1 to 12.0 and 13.5% on day 106; and in Zone B it ranged from 35 and 47% on day 1 to 3 and 7% on day 106, for the urine + fertiliser and urine only treatments, respectively. Total $^{15}$N recovery in the urine + fertiliser treatment was greater (p < 0.001) in Zone A compared to Zones B and C on all sample times except 43; and greater (p < 0.001) in Zone B compared to Zone C on days 1 to 14 (Figure 5.35a). In the urine only treatment, total $^{15}$N recovery was greater in Zone A compared to Zone B and C on all days except 1, 8 and 43; and was greater (p < 0.05) in Zones A and B compared to C on days 1, 8 and 14.

In the 7.5-20 cm depth, total $^{15}$N recovery in Zone A ranged from 37 and 7.0% on day 1 to 4.0 and 5.0% on day 106; and in Zone B it ranged from 4.0 and 15% on day 1 to 0.7 and 3% on day 106, for the urine + fertiliser and urine only treatments, respectively. In the urine + fertiliser treatment, soil total $^{15}$N recovery was greater (p < 0.001) in Zone A compared to Zones B and C on all sample times except 1 and 106. Zone B did not differ from Zone C on any sampling day (Figure 5.35b). In the urine only treatment, total $^{15}$N recovery was greater (p < 0.001) in Zone A compared to Zones B and C on days 14 and 21 only. There were no other differences in total $^{15}$N recovery between zones except on day 106 where Zone A was marginally greater (p < 0.05) than Zone C (Figure 5.35d). On day 63, an increase in soil total $^{15}$N recovery in Zone C, was due to a single outlier in one of the four replicates, and was not statistically significant.

There was no effect of fertiliser and no interaction between the urine and fertiliser treatments on soil total $^{15}$N recovery on any sampling day at either depth.
Figure 5.35 Soil total $^{15}$N recovery from day 0 to 106 from the urine + fertiliser treatment at (a) 0-7.5 cm and (b) 7.5-20 cm depths and from the urine only treatment at (c) 0-7.5 cm and (d) 7.5-20 cm depths. Error bars = LSD (5%), n = 4.

5.3.3.9 Microbial biomass N

Plot scale

In the 0-7.5 cm depth, at the whole plot scale, soil microbial biomass N ranged from 140-200, 122-312, 180-214 and 81-212 $\mu$g N g$^{-1}$ soil in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively. The greatest variation in soil microbial biomass N, between treatments, occurred within the first 21 days following treatment application where the maximum values were 78, 97, 234 and 58 $\mu$g N g$^{-1}$ soil on days 1, 8, 14 and 21, respectively, (Figure 5.36a). For the remainder of the experiment, the range in soil microbial biomass N did not exceed 32 $\mu$g N g$^{-1}$ soil. There was a urine effect on day 1, where microbial biomass N in the fertiliser only treatment was greater (p<0.05) than the urine treatments; and day 14, where microbial biomass N in the urine only treatment was greater (p < 0.05) than all other treatments (Figure 5.36a). From day 21 onwards, microbial biomass N did not differ amongst treatments.
In the 7.5-20 cm depth, soil microbial biomass N ranged from 60-100, 56-112, 57-91 and 45-82 µg N g⁻¹ soil in the urine + fertiliser, urine only, fertiliser only and control treatments, respectively. Variation in soil microbial biomass N between treatments was not as great as in the first 21 days as in the 0-7.5 cm depth with a maximum values of 35, 54, 43 and 34 µg N g⁻¹ soil on days 1, 8, 14 and 21, respectively (Figure 5.36b). There was a urine effect on day 8, where the microbial biomass N from the urine only treatment increased above all other treatments (p < 0.05); and on days 43, 63 and 106 where the microbial biomass N was greater (p < 0.05) in the urine treatments than the non-urine treatments (Figure 5.36b).

There was no fertiliser effect or interaction between the urine and fertiliser treatments on microbial biomass N at the plot scale.

Figure 5.36  Soil microbial biomass N (µg g⁻¹ soil) at the plot scale at (a) 0-7.5 cm depth and (b) 7.5-20 cm depth for treatments (U+F+) urine + fertiliser; (U+F0) urine only; (U0F+) fertiliser only and (U0F0) control from days 0 to 106. Error = LSD (5%), n = 4. Note the y axes differ.

Zone scale

In the 0-7.5 cm depth, at the zone scale microbial biomass N in the urine + fertiliser treatment did not differ significantly between zones B and C at any time during the experiment; however, microbial biomass N in Zone A was less (p < 0.05) than that in Zones B and C on days 1, 8, 21 and 43 (Figure 5.37a). In the urine only treatment, there was no significant difference in soil microbial biomass between zones, with the exception
of day 14, where microbial biomass N in Zone A was greater (p < 0.05) than in Zones B and C, reaching 629 µg N g⁻¹ soil (Figure 5.37b). This was considerably greater than any other microbial biomass N measurement during the experiment, and was due to a single outlier in one of the four replicates. In the non-urine treatments, there was no fertiliser effect, or urine and fertiliser interaction on soil microbial biomass N, and no significant difference between zones (Figure 5.37c & Figure 5.37d).

In the 7.5-20 cm depth, microbial biomass N in the urine + fertiliser treatment did not differ significantly between zones A, B or C at any time during the experiment (Figure 5.38a). In the urine only treatment, on day 8 microbial biomass N in Zone A and B was greater (p < 0.05) than that in Zone C, then on day 14, microbial biomass N in Zone A declined by 142 µg N g⁻¹ soil and was less (p < 0.05) than Zone B, but not Zone C. After this, microbial biomass N did not differ between any of the zones (Figure 5.38b). In both non-urine treatments there was no difference between zones at any time during the
experiment (Figure 5.38c & Figure 5.38d). There was no urine or fertiliser effects, and no interaction between the urine and fertiliser treatments on soil microbial biomass N at this depth.

![Graph](attachment:graph.png)

**Figure 5.38** Soil microbial biomass N (µg N g⁻¹ soil) at 7.5-20 cm depth from Zones A, B and C for treatments (a) urine + fertiliser; (b) urine only; (c) fertiliser only and (d) control, from day 0 to 106. Error = LSD (5%), n = 4.

### 5.3.3.10 Microbial biomass ¹⁵N recovery

Due to budget constraints, microbial biomass ¹⁵N was measured only in the top 0-7.5 cm depth. The ¹⁵N diffusion process was only performed on oxidised K₂SO₄ extract sub-samples that had sufficiently high N concentrations to enable detection on the IRMS, and as such, no diffusions were carried out on samples from Zone B from day 14 onwards, or Zone C from any day so ¹⁵N recovery in these instances was considered negligible and given values of zero. Furthermore, N concentrations were not sufficiently high in all replicates to facilitate ¹⁵N diffusion of each treatment, in each zone, on each day. This has therefore more-than-likely introduced an element of statistical bias to the results. This does not detract from the purpose of measuring microbial biomass ¹⁵N, which was to
gain a better understanding of the overall amounts of urinary deposited $^{15}$N recovered as soil microbial biomass N over time, which, as reported below, were negligible (Figure 5.39 and Figure 5.40).

**Plot scale**

Recovery of urinary $^{15}$N in the soil microbial biomass at the plot scale was very low, ranging from $< 0.001$-$0.0032\%$ in the urine + fertiliser treatment and $< 0.001$-$0.0045\%$ in the urine only treatment, and decreased with time in both treatments (Figure 5.39).

While microbial biomass $^{15}$N recovery was greater ($p < 0.05$) in the urine only treatment on days 8 and 14, there was no statistical difference in $^{15}$N recovery between treatments on any other day (Figure 5.39). There was no fertiliser effect, nor any interaction between the urine and fertiliser treatments on microbial biomass $^{15}$N recovery at the plot scale on any sampling day.

![Figure 5.39 Soil microbial biomass $^{15}$N recovery (%)](image)

**Zone scale**

Microbial biomass $^{15}$N recovery in Zone A ranged from $< 0.001$-$0.0014\%$ and $< 0.001$-$0.0016\%$; and in Zone B ranged from $0.0030$-$0.0056\%$, and $0.0052$-$0.0074\%$ in the urine + fertiliser and urine only treatments, respectively (Figure 5.40). Total microbial biomass
15N recovery (sum of Zones A and B) at the end of the experiment was 0.0009 and 0.0004% in the urine + fertiliser and urine only treatments respectively.

Although microbial biomass 15N recovery in Zone A was measured over a longer time period than zone B, 15N recovery was greater (p < 0.001) in Zone B on days 1 and 14 in the urine + fertiliser treatment, and on days 1, 8 and 14 in the urine only treatment (Figure 5.40). There was a slight decrease in microbial biomass 15N recovery in Zone A over time, and no definitive temporal trend in Zone B (Figure 5.40). There was no fertiliser effect, nor any interaction between the urine and fertiliser treatments on any sampling day.

![Figure 5.40 Soil microbial biomass 15N recovery (%) at the 7.5-20 cm depth from Zones A and B for treatments (a) urine + fertiliser and (b) urine only, from day 0 to 43. Error = LSD (5%), n = 4.](image-url)
5.4 Discussion

5.4.1 Pasture

5.4.1.1 Pasture $^{15}$N recovery

Pasture $^{15}$N recovery values in the literature vary widely from 11% (Clough et al., 1996) to 65% (Leterme et al., 2003), and the mean values in the current study (50-52%) are at the higher end of the range. This is presumably because the urine was applied during spring, when conditions are generally favourable for pasture growth, and the greatest pasture responses are observed (Ball and Field, 1982). The pasture $^{15}$N recovery results were similar to those reported by Decau et al. (2003) and Leterme et al. (2003), where recoveries ranged from 30-65% from urine applied in spring or summer. The lower pasture $^{15}$N recoveries following urine application range from 12-47% (Fraser et al., 1994; Williams and Haynes, 1994; Clough et al., 1996; Clough et al., 1998b; Di et al., 2002; Silva et al., 2005) and occur when urine is winter or autumn applied, with lower recoveries due to greater leaching and denitrification losses of N.

The high summer rainfall observed in December 2011 and January 2012 was uncharacteristic and unexpected. The field trial was designed with the urine and fertiliser treatments applied in mid-spring to minimise N losses via leaching and denitrification, which have been reported to be highest during the wetter colder months where higher rainfall, reduced evapotranspiration and shorter day length result in reduced pasture uptake, drainage and overall wetter soil conditions (Cameron et al., 2013). Although speculative, perhaps the recovery in the pasture and the soil pools would have been higher if summer rainfall and subsequent drainage had been lower.

Urinary $^{15}$N recovery in the pasture was not limited to Zone A. Up to 18% of the urinary N was recovered in Zone B and up to 4.2% was recovered in Zone C, resulting in a total of up to 22% of urinary N being accounted for in the pasture outside the wetted area of the urine patch. The greatest proportion of $^{15}$N was recovered in Zone A and decreased with increasing distance from the urine patch (Figure 5.21). This was expected because the urine application, and thus the total N loading was confined to Zone A. The recovery of $^{15}$N in Zone B was expected due to the possibility of lateral movement of the urine on application, which is discussed later; however, the recovery of nearly 5% of the urinary N in Zone C, up to 0.5 m from the outside edge of the urine patch, was surprising, because
visual urine patch effects on pasture growth were only observed in Zones A and B, and not Zone C during the experiment (Figure 5.41).

Although the recovery of \(^{15}\)N in the pasture was significant in zones B and C, the actual \(^{15}\)N uptake on a unit area (per m\(^2\)) basis was considerably smaller in Zones B and C than in the wetted area of Zone A (Figure 5.21a). This was due to the greater area in Zones B and C (0.67 and 1.02 m\(^2\), respectively) compared to that in Zone A (0.28 m\(^2\)), and therefore the greater amount of pasture harvested from these zones. However, this does not detract from the key finding that urinary \(^{15}\)N is being utilised by plants at a radial distance of up to 0.8 m from the centre of a urine patch.

The recovery of \(^{15}\)N and soil background N in the pasture declined rapidly with distance from the urine patch from Zone A to Zone B (Figure 5.21). It is unknown whether this decrease in urinary N uptake with distance occurs linearly (as inferred by Figure 5.21) or if it is characterised by an exponential decline. Further work is therefore required to better define the gradient of urinary N uptake by pasture with distance from the wetted area of a urine patch.

The results suggest that the actual effective area of a urine patch (based on pasture response) is at least 1.97 m\(^2\) (0.28 + 0.67 + 1.02 m\(^2\)) which is about six times the wetted area. However, due to the fact that > 95% of the \(^{15}\)N was taken up by pasture in Zones A and B, and that \(^{15}\)N uptake by pasture on a per unit area basis was very small in Zone C, it might be argued that the key effective area need not include Zone C, which would essentially halve the effective area to 0.95 m\(^2\), making it three times the wetted area. However, despite this, the \(^{15}\)N recovery data is unequivocal evidence that pasture as far out as Zone C is utilising the urinary N from the wetted area, therefore, although its contribution to the total \(^{15}\)N uptake is small, it could still be considered to contribute to the effective area. Considering that previous research shows up to 29% of the grazed paddock area can be covered in urine patches per year (Dennis et al., 2011; Moir et al., 2011); if 22% of urinary N in every urine patch is being utilised by an area of pasture that is three or six times the wetted area of the urine patch, this may have considerable implications for not only the area of grazed farmland affected by urine, but also for the estimation and modelling of plant uptake from urine patches at the paddock or farm scale.
The uptake of background (non-$^{15}$N) N on a unit area (per m²) basis suggests that as well as taking up urinary N, the application of urine stimulated even larger amounts of plant uptake from the non-labelled background soil N pool (Figure 5.21b). This is termed a ‘priming effect’ and is most likely a result of enhanced solubility of soil organic matter following urine application, resulting in enhanced soil microbial activity and thus increased mineralisation of organic and microbial N, ultimately manifesting as increased unlabelled N uptake by the pasture. The N uptake from the background soil N pools as a proportion of the total uptake was around 70, 85 and ≥90% in Zones A, B and C, respectively. These results are similar or within the same range as other studies that have measured plant $^{15}$N uptake as a proportion of the total N uptake under $^{15}$N amended urine. For example, similar N uptake was reported in a urinary $^{15}$N recovery study by Decau et al. (2003) where total background N uptake by plants ranged from 70-98% of the total plant N uptake. Plant N uptake from the background soil N pool was also within the range of results reported by Fraser et al. (1994) who reported plant uptake from the background N pool to range from 33-93% of total N uptake. Silva et al. (2005) also reported N uptake from the background soil N pool of up to 75% which was within the same range as those reported here, however, this study also reported proportions as low as 10%. The effects of priming are arguably short term as the increased mineralisation of background soil N is followed by increased immobilisation (Jenkinson et al., 1985) and have been shown to extend beyond plant uptake effects to NO$_3$-N leaching (Wachendorf et al., 2005) and N$_2$O emissions (via soil C priming) (Clough and Kelliher, 2005).
Lateral surface movement of the urinary N from Zone A to the inside edge of Zone B at the time of treatment application cannot be ruled out; however, every precaution was taken to ensure this did not happen. The urine, and water applied to wash it in were applied very slowly over a number of hours to ensure the liquid soaked exclusively into the soil in Zone A, and no observations of runoff were noted at the time of treatment application. Although this is not how cattle urine is deposited in the field, the objective of this study was to quantify the extent of the effective area of a urine patch, therefore, it was considered more important to ensure that runoff did not occur.

Alternatively, subsurface movement of urine through laterally connected macropores could have occurred (Williams et al., 1990a); or the lateral diffusion of NO$_3^-$-N along a concentration gradient (Wild, 1972). This latter suggestion is not likely to have contributed significantly to the pasture $^{15}$N recovery in Zones B and C because the diffusion rate of NO$_3^-$-N in the soil is low at only $\sim$1 mm d$^{-1}$ (McLaren and Cameron, 1996). This translates to a time of 300 and 550 days for urinary NO$_3^-$-N to diffuse from the centre of Zone A to the inside edges of Zone B and Zone C, respectively and (a) the experiment was completed by this time and (b), the NO$_3^-$-N would most likely have been taken up by plant roots or microbes early on in its diffusive transit.

Ammonia volatilised from the soil following $^{15}$N enriched urine application can be directly absorbed through the stomata of plants. This occurs when the mole fraction of NH$_3$ in the atmosphere is higher than that which is above the mesophyll cell walls in the substomatal cavity. Conversely, when the opposite is true, evolution of NH$_3$ from plant leaves occurs (Farquhar et al., 1980; Sommer and Jensen, 1991; Schjoerring and Mattsson, 2001; van Hove et al., 2002). A number of studies have assessed NH$_3$ absorption and emission from crop plant canopies such as maize (Denmead et al., 1982; Harper et al., 2000), sugarcane (Denmead et al., 2008), rapeseed, barley, wheat and peas (Schjoerring and Mattsson, 2001). However, there is little known about the extent of volatilised NH$_3$ that is re-deposited and absorbed by pasture following urine deposition, or the distances NH$_3$ travels from the source (i.e. the urine patch) (Ross and Jarvis, 2001). Sommer and Jensen (1991) measured foliar absorption of ryegrass at various distances from a dairy farm dung yard and measured deposition of 3.0 and 0.7 g N m$^{-2}$, 10 m and 130 m from the source, respectively. Ross and Jarvis (2001) measured NH$_3$ emission and deposition from ryegrass swards affected by urine using a wind tunnel, and found that 20-60% of NH$_3$ emitted was deposited within a distance of 2 m.
These studies show NH$_3$ deposition and absorption by nearby pasture can be substantial, however, in the current study, minimal emissions of NH$_3$ from the soil were expected following treatment application because 10 mm water was applied to immediately wash in the treatments. Other studies have shown this to be an effective means to suppress NH$_3$ volatilisation (Black et al., 1987; Fraser et al., 1994; Clough et al., 1996), therefore plant absorption of volatilised NH$_3$ would also be expected to be minimal. However, NH$_3$ losses from plant stomata are increased under conditions of large root N absorption and high N concentrations in foliage (Schjoerring and Mattsson, 2001); therefore it is possible that urinary $^{15}$N was released from the affected pasture in Zone A, and some of it was re-absorbed by pasture in Zones B and C, or outside the plot altogether. The primary factors affecting this include wind-speed and the density and height of the canopy cover (Denmead et al., 1982; Asman, 1998). Pasture re-absorption of released NH$_4^+$-N is greater under low wind speeds and dense pasture conditions. More research in this area is required to determine the role that NH$_3$ absorption and emission from pastoral systems play in the fate of N from a urine patch.

Another possibility is the transmission of $^{15}$N enriched NH$_3$ gas through interconnected soil pores and the conversion of this to NH$_4^+$ upon contact with the comparatively acid conditions outside the highly alkaline wetted soil area of the urine patch. Plant uptake of the $^{15}$NH$_4^+$ could then subsequently have occurred. Pasture $^{15}$N uptake by this means would most likely have occurred in Zone B, because the NH$_3$ gas would be transformed rapidly and would therefore not be transmitted as far as Zone C.

Root extension from Zones B and C into the ‘high N’ zone, i.e. Zone A, and subsequent exploitation of the localised supply of N is the most likely explanation for the pasture $^{15}$N recovery measured in Zones B and C. This is supported by the fact that there was no increase in soil inorganic N concentrations, nor any soil $^{15}$N recovered from Zone C, yet nearly 5% of the urinary $^{15}$N was recovered in the pasture. Furthermore the increase in soil inorganic N concentrations and soil $^{15}$N recovery in Zone B were very low and short-lived, relative to Zone A (Figure 5.25, Figure 5.27 and Figure 5.32), yet up to 18% of the urinary N was recovered in Zone B pasture, thus it is unlikely the majority of $^{15}$N utilised by the pasture came from the soil in Zone B. As a coping mechanism for the localised supply of nutrients in soil, plant roots are flexible (termed root plasticity), and respond to nutrient rich areas by proliferating (or “foraging”) and enhancing their physiological capability for ion uptake of nutrients (Whitehead, 1986; Robinson et al., 1999; Hodge,
Nitrogen uptake in particular has been strongly associated with plant root proliferation in inorganic N-rich patches in otherwise N deficient soils (Robinson et al., 1999). The term root proliferation specifically refers to the initiation of new lateral branching (Hodge, 2004); however, other morphological responses include enhanced root production and the elongation of individual roots. Physiologically, when plants have a rich supply of nutrients (i.e. NO$_3^-$-N in a urine patch), their uptake increases two to three-fold per unit area of root (Robinson, 1994). Hodge, (2004) reported that the extent of plant root proliferation depends primarily on (a) the plant demand for N, (b) the mobility of N within the plant, and (c) the concentration of available N relative to background soil fertility. A urine patch consists of a very high N loading, in this case 800 kg N ha$^{-1}$ was applied over a small area. In order to benefit from this N-rich patch, the capacity for N uptake by the plants in and around the affected area increased, as was observed in the harvest data (Figure 5.14 and Figure 5.17), and the root proliferation and extension of laterals from Zone B and as far out as Zone C essentially tapped into the readily available inorganic N source applied to Zone A. Luxury uptake of N, where excess N taken up by pasture is stored as NO$_3^-$ or amides (Whitehead, 1995), also played a role here as illustrated by the increased pasture N content in the urine-affected treatments (Figure 5.12).

The considerable urinary $^{15}$N uptake (up to 22% of the urinary N) from outside the ‘wetted area’ of the urine patch has implications for lysimeter studies such as that detailed in Chapter 3. In studies investigating the effect of urine patches on NO$_3^-$-N leaching using similar sized lysimeters (0.5 m diameter x 0.7 m depth), it is often not specified whether the urine was applied over the entire surface area of the lysimeter or not, e.g. Silva et al. (1999), Di et al. (2002), Silva et al. (2005), Di and Cameron, (2002a), and Menneer et al. (2008). Still, the surface area of a 0.5 m diameter lysimeter (0.19 m$^2$) is within the range of the average wetted area of a urine patch, 0.16 to 0.49 m$^2$ (Haynes and Williams, 1993), so it seems sensible that urine treatments applied to lysimeters cover this entire area. However, the lysimeter casing creates a physical barrier between the plant root systems inside and outside the lysimeter, which is not present under field conditions. This means that in a lysimeter experiment pasture in the ‘effective area’ (i.e. outside the lysimeter) does not proliferate and utilise urinary N from the urine patch like they have been observed to do in the field. This may effectively mean that overall, less urinary N can be taken up by pasture because it is only available to the pasture within the confines
of the lysimeter casing. Consequently, there may be higher leaching and/or gaseous losses observed from a lysimeter than would occur under an identical field situation. In turn, this has further implications for the ongoing development of farm-scale agricultural nutrient models (e.g. process based model APSIM) which simulate the flow and fate of nutrients throughout a system (including the simulation of NO$_3^-$-N leaching from pastoral systems). The predictions of such models are becoming increasingly useful as decision support tools for farmers and policy makers as they can estimate nutrient dynamics where there is little to no capacity for actual observations. However, these models are often reliant on empirical data for validation and/or calibration, therefore it is essential that this data is as accurate as possible, particularly if the outputs of such models are going to be used as justification for farm management decisions, or the implementation of new regional policies.

These findings raise further questions on N leaching and pasture uptake dynamics from lysimeter studies. Plant root uptake of N is competitive, so in the absence of plant roots outside the wetted area, the plants within the lysimeter may be able to take up more N. In a lysimeter study where urine is applied to the entire surface area, if the pasture roots are confined within the lysimeter casing, are these exposed to greater amounts of available N than in the field, resulting in higher yield and N uptake in lysimeters (i.e. more luxury N uptake) compared to the field? A comparison of the yield and N uptake results of the current study with those of the lysimeter study over the same time period detailed in Chapter 3 suggests this may be the case. The pasture yield data between the two studies was very similar (Figure 3.21a and Figure 5.7), but the N uptake in the current study (Figure 5.14) was slightly lower than in the lysimeter study over the same time period (Figure 3.23a). Determining the extent of the discrepancy (if any) of the fate of N between lysimeters that have and have not accounted for pasture uptake outside the wetted area of a urine patch was outside the scope of the lysimeter study (Chapter 3), however, it is an important area for future research.

Recovery of $^{15}$N in the pasture was greatest in the first harvest and declined with increasing number of harvests. The $^{15}$N recovery (in Zones A and B) declined below 1% after 135 days (Figure 5.18). Similar patterns were observed by Di et al. (2002) and Clough et al. (1998a), who also recovered the majority of $^{15}$N in the pasture by day 130, although the total $^{15}$N recovered was considerably less (22 to 34%) than that measured in this study.
Few studies have presented pasture $^{15}$N recovery data over time, most tend to present a total cumulative recovery over the entire experiment (Fraser et al., 1994; Williams and Haynes, 1994; Leterme et al., 2003; Silva et al., 2005). Furthermore, in these previous studies, the first pasture harvests were generally at least three weeks following treatment application, therefore little is known about the dynamics of pasture uptake of urinary $N$ within the first days and weeks after urine application. Six pasture cuttings were taken within the first 50 days of this study and analysed for their $^{15}$N enrichment. The results showed that uptake of the urinary $N$ ($^{15}$N) had occurred in Zones A and B by day 1 (Figure 5.22). Pasture $^{15}$N enrichment increased rapidly until day 10, after which it plateaued, (or slightly decreased in the case of Zone B). This was probably a result of optimal plant growth conditions at that time, i.e. warm temperatures, long daylight hours, a plentiful source of $N$, available water (used to wash in the treatments), and the water in the urine itself. Perhaps if the urine had been applied in late autumn or winter, the plant response may have been slower. The urine’s $^{15}$N enrichment was 5 atom%, so $\sim 70\%$ of the plant $N$ was urine derived with the remaining $\sim 30\%$ derived from the antecedent soil $N$ pool.

5.4.1.2 Pasture $N$ uptake

Pasture $N$ uptake followed a similar pattern to DM yield both temporally and cumulatively. There was an increase in the $N$ content of pasture in the urine-affected treatments in the first three harvests, but after this it returned to within control values, indicating that DM yield was a better determinant of $N$ uptake than the $N$ content of the pasture. The decline in pasture $N$ content, across all zones of all treatments, on day 133 (Figure 5.11 and Figure 5.12) could be due to the six week dry period between days 85 and 133 (Figure 5.3). Generally, in summer months when water and available $N$ are limited, $N$ is recycled within the plant as an energy conservation mechanism. Rather than taking up $N$ from the soil via the roots, new plant tissue takes $N$ from the older plant tissue, essentially diluting the overall $N$ content in the plant (Pate, 1973; Pate and Farquhar, 1987).

By day 165, the pasture $N$ contents had increased, yet this did not coincide with any major increase or decrease in pasture yield (Figure 5.7). This increase was presumably a result of the more frequent rainfall between days 133 and 165 following the dry period mentioned in the above paragraph. This re-wetting of the soil would have resulted in a
flush of mineralisation and plant N uptake (a small but non-significant increase in N uptake occurred on day 165 in most zones of all treatments (Figure 5.12)). Furthermore, because the soil moisture limitation had been relieved, the pasture may have taken up more N from the soil, rather than relying on the N recycling described above to support new growth.

In the urine treatments, pasture N uptake was greatest in Zone A, and decreased with increasing distance from the urine patch. After day 85, the urine effect on pasture N uptake disappeared (Figure 5.7). Between days 58 and 85 there was a considerable increase in pasture N uptake in Zones B and C from all treatments, while in Zone A, pasture N either plateaued or slightly declined (Figure 5.7). The increase in pasture N uptake in Zones B and C was likely due to the higher than normal rainfall in December 2011 (224 mm) and January 2012 (99 mm) following a very dry November (Figure 5.3). The plots received 218 mm of rain between days 58 and 85. The soil re-wetting effect may have resulted in a flush of mineralisation (Haynes, 1986b) due to the recovery of microbial communities after the dry period and the warm summer temperatures that were non-limiting to microbial growth. The abundance of water and mineralised N potentially resulted in considerable pasture growth and N uptake further aided by the warm temperatures and longer sunlight hours at this time of year.

The subsequent decline in pasture N uptake on day 106 in Zones A, B and C of all treatments suggests any remaining NO$_3^-$-N in the soil had been lost from the system. Some of this was likely due to previous plant uptake, but also, the large rainfall events resulting in drainage would have leached any unutilised NO$_3^-$-N below the plant root zone. Although drainage and N leaching were not measured in this experiment, they were measured from the same soil type in the lysimeters detailed in Chapter 3, which were located about 7 km from this field trial. There was an average of 121 mm drainage recorded from the 36 lysimeters between 1 Dec 2011 and 11 Jan 2012. Evidently, drainage and leaching of inorganic NO$_3^-$-N and potentially dissolved organic N (DON) would have occurred at the field site, however, the amounts lost remain unquantified. Lastly, an increase in nitrification following the above-mentioned flush of mineralisation may have resulted in a pulse of N$_2$O emissions. The large rainfall events (up to 62 mm in one day) would have created temporary anaerobic conditions in the soil, resulting in N$_2$O and N$_2$ emissions via denitrification. It is possible that with the warm soil temperatures and, in the case of the urine treatments, the comparatively high levels of C, denitrification
could represent a significant loss of N from the system. However, as with the leaching, the gaseous N loss was not quantified.

In the non-urine treatments (fertiliser only and control), on day 58 there was increased pasture N uptake in Zone A only (Figure 5.14). This is not likely to be a fertiliser effect because (a) it occurred in both the fertiliser only and control treatments; and (b) in the fertiliser only treatment there was no fertiliser effect observed in the previous harvest (day 32) nor any of the following harvests. The fact this increase in N uptake occurred in Zone A of the control treatments suggests that the water applied to all treatments (including controls), to wash in the applied N and prevent NH3 emissions (Black et al., 1987) played a role, as this was the only point of difference between Zone A, and Zones B and C. Prior to day 58, there had been very little rain, then but on day 47 there was a 12 mm rainfall event. Perhaps this, with antecedent soil moisture from the 10 mm water applied to Zone A on Day 0 was enough to result in a flush of mineralisation, and the observed increase in pasture N uptake over the next 11 days. It is most likely there was still a residual mineralisation effect in Zones B and C following the 12 mm rainfall on day 47, but perhaps, due to the drier antecedent soil conditions, the benefits of this to the pasture were delayed and not observed until the following harvest on day 106.

5.4.2 Soil

5.4.2.1 Soil 15N recovery

Inorganic soil 15N recovery was as expected, greatest on day 1 and declined over time (Figure 5.18). Up to 25 and 40% of urinary N was recovered in Zone B on day 1 in the urine + fertiliser and urine only treatments, respectively, indicating that a considerable amount of urinary N moved from Zone A into Zone B in the 24 hours after application. On day 1, in Zone A, the recovery of 15N applied as inorganic N in the soil (both depths) was 74 and 48% (±39) in the urine + fertiliser and urine only treatments, respectively (Figure 5.32). The pasture cutting data shows that a small amount of the missing 15N was taken up by pasture on day 1 (Figure 5.22), and some 15N would have been emitted as N2O and/or N2. Ammonia emissions should have been negligible due to the water applied after the treatment application (Black et al., 1987). A further sink for 15N was incorporation into the microbial biomass, as previous work has shown that soil microbes are strong competitors for inorganic N in the short term (up to 24 hours) after N application (Jackson et al., 1989; Hodge et al., 2000; Inselsbacher et al., 2010). The
decline of $^{15}$N recovery in the soil with time can be largely attributed to increasing cumulative plant uptake of the $^{15}$N over time. Microbial biomass did not increase with time so was not a large sink for N relative to the pasture, however, the nature of the bulk measurements of microbial analysis may have resulted in some microbial N uptake going undetected. Furthermore, the turnover rate of pasture is much slower than that of microbes, enhancing the capacity for plants to outcompete microbes for available N over extended periods of time (Inselsbacher et al., 2010).

The latest date that soil inorganic $^{15}$N diffusions were carried out was 29 Nov 2011 (day 43) and on this day there was 22 and 12.2% of the $^{15}$N applied recovered as inorganic N in Zone A (both depths) in the urine + fertiliser and urine only treatments, respectively. The soil inorganic $^{15}$N recovery in Zone B had returned to zero by day 14 in both treatments. After this date, the soil $\text{NH}_4^+$-N and $\text{NO}_3^-$-N concentrations were too low to enable $^{15}$N diffusions, therefore it is probable that the high rainfall and drainage in December 2011 and January 2012 leached any remaining $\text{NO}_3^-$-$^{15}$N below the measured depth of 20 cm. No soil $^{15}$N was recovered in Zone C (Figure 5.32 and Figure 5.33) inferring that the entire pasture $^{15}$N uptake in Zone C was due to pasture root proliferation, not uptake of laterally displaced urinary $\text{NO}_3^-$-N. It also supports the earlier suggestion that the majority of plant uptake of urinary $^{15}$N observed in Zone B is also a result of pasture root proliferation.

The $^{15}$N recovered in the organic fraction was calculated as the difference between the total soil $^{15}$N and inorganic $^{15}$N recoveries. In the first few measurements, up to 18% of urinary N was recovered as soil organic $^{15}$N. It was initially thought that the primary source of the organic $^{15}$N recovered within the first few weeks was microbially immobilised N because there was not time for $^{15}$N enriched plant organic material to contribute to soil $^{15}$N at this stage in the experiment, however, the $^{15}$N recovery measured in the microbial biomass was < 0.001% (Section 5.3.3.10), and therefore contradicted this supposition. Ledgard et al. (1988) measured fertiliser microbial biomass $^{15}$N recovery that was similar to the $^{15}$N recovery from the soil organic fraction measured in the current study. There was no statistical difference between the organic $^{15}$N recovered in in Zones A and B of the urine + fertiliser and urine only treatments on days 1 and 8, where organic $^{15}$N recovery was highest. This is because there was a plentiful source of $^{15}$N enriched urinary N in both zones at the beginning of the experiment for rapid immobilisation. However, as time went on, the organic $^{15}$N recovery in Zone B declined while remaining
higher in Zone A (Figure 5.33). The substantial decrease in organic $^{15}$N recovery in Zone A in both treatments on day 43 is probably due to a lack of available C to sustain continued microbial growth (Hodge et al., 2000; Inselsbacher et al., 2010) coupled with increasingly dry soil conditions at this time.

### 5.4.2.2 Inorganic soil N

The peak soil NH$_4^+$-N and NO$_3^-$-N concentrations were within the range of other previously reported values under cattle urine (Jackson et al., 1989; Williams and Haynes, 1994; Menneer et al., 2008; Orwin et al., 2009; Orwin et al., 2010).

Interestingly, the difference in soil NH$_4^+$-N in Zone A of the urine + fertiliser and urine only treatments was 140 $\mu$g NH$_4^+$-N kg$^{-1}$ soil, which was nearly three times that measured in the fertiliser only treatment (Figure 5.25). This suggests that the additional N in the fertiliser + urine treatment stimulated (i.e. primed) the mineralisation of background soil N, and that this did not occur to the same extent in the presence of urine only. This might also explain the slightly (though not statistically significant) increased pasture N uptake in the urine + fertiliser treatment, compared to the urine only treatment (Figure 5.14). The same observation occurred in the soil NO$_3^-$-N, and expectedly so because much of the additional NH$_4^+$-N observed in the urine + fertiliser treatment was rapidly nitrified to NO$_3^-$-N.

The very small and short-lived increase in soil NO$_3^-$-N observed in in Zones B and C in the 0-7.5 cm depth of the urine-affected treatments infers that very little treatment-associated N moved laterally in the soil from Zone A outwards. The fact that small amounts of NH$_4^+$-N and NO$_3^-$-N were measured in Zone B very early in the experiment, days 1 and 8, respectively, (Figure 5.25 and Figure 5.27) indicates that the source of inorganic N in Zone B was due to either (a) surface runoff of urine at the time of application from Zone A into Zone B; and/or (b) subsurface flow of urine at the time of application through laterally connected macropores. Studies have shown that substantial preferential flow occurs through macropores in the topsoil following a urination event because the urine application rate exceeds the soil water matrix flow (infiltration rate) (Williams et al., 1990b). Preferential flow occurs through displacement if the soil is initially moist or at field capacity, or through “short-circuiting”, i.e. the rapid free movement of water through air-filled pore spaces in unsaturated soil. Short-circuiting is associated with the lateral movement of water (or urine) into the adjacent unsaturated soil.
through a series of laterally connected macropores (Bouma, 1981). The extent of this is determined by the degree to which lateral movement is the path of least resistance and is a factor of the urine application rate, hydraulic conductivity and the antecedent soil moisture (Bouma and Anderson, 1977; Bouma, 1981). This is the most likely explanation for the soil NH$_4^+$-N and NO$_3^-$-N observed in Zone B so soon after urine application. Surface runoff of applied urine was a possibility but care was taken to avoid this and it was not observed.

**5.4.2.3 Total and organic soil N**

There was a urine effect with zone where the total soil N in Zone A was greater than in Zone B and C in the urine treatments until day 43 (Figure 5.31). This was probably attributable to the large increase in the inorganic N fraction as a result of the urine treatment applications, which is supported by the fact that the soil NO$_3^-$-N peaks in the same treatments also returned to control levels after day 43.

**5.4.2.4 Microbial biomass N**

Microbial biomass N tended to be lower in Zone A compared to Zones B and C in the urine + fertiliser treatment up until day 63. In the urine only treatment there was no apparent difference in microbial biomass N with zone. The lower microbial biomass N in Zone A of the urine + fertiliser treatment was possibly due to osmotic stress as a result of the increased salt concentrations following urine application (Wachendorf et al., 2005; Orwin et al., 2010), however, the same trend was not observed in the urine only treatment, thus contradicting this interpretation. Another possibility could be that the higher N loading applied to the urine + fertiliser treatment resulted in higher soil NH$_4^+$-N concentrations, and subsequently an osmotic shock effect on the microbial community (Norton and Stark, 2011). Although greater soil NH$_4^+$-N concentrations were observed in the urine + fertiliser treatment compared to the urine only treatment, it is unlikely that the additional 35 kg N ha$^{-1}$ applied as fertiliser would initiate such microbial stress amongst the much larger N loading of 800 kg N ha$^{-1}$ applied as urine. Other studies (e.g. Orwin et al. (2010)) observed a sharp increase in microbial biomass as a result of urine application, followed by a sharp decrease, with recovery after about 45 days. In this study, the microbial biomass N appeared to recover after 63 days (Figure 5.37a).

Although there were no consistent trends by zone or with treatments, there was a trend where in all zones of all treatments, at both depths, the microbial biomass N appeared to
settle out after day 43. This suggests that the N supply from the treatments, along with climatic variables affected the soil microbial biomass N. Until about day 50, there was very little rainfall, and with increasing temperatures and evapotranspiration over this time, soil moisture decreased resulting in decreased microbial activity. The available C source supplied with urine application would have also decreased, further limiting microbial activity. The rainfall after day 50 would have resulted in a flush of mineralisation, including the re-mineralisation of N previously immobilised in the microbial biomass. There are potentially two main reasons why no increase in microbial biomass N occurred after this time; (1) there was little inorganic NO$_3^-$-N remaining in the soil to be immobilised, and that which was would have been leached by the drainage that occurred in Dec 2011 and Jan 2012 (~days 60 to 88), and (2) conditions were very good for plant uptake of inorganic N and the combination of reduced microbial population and re-mineralised N that was previously in the microbial biomass provided a competitive advantage to the plants. This corroborates results from other studies, which have found that even though soil microbial biomass can be a successful competitor for N over the short term (hours), plants, have a longer lifespan relative to microbes, and can continue to compete for N over time taking advantage of microbial turnover and store N in plant tissue (Jackson et al., 1989; Kaye and Hart, 1997; Hodge et al., 2000; Harrison et al., 2008; Inselsbacher et al., 2010).

The recovery of $^{15}$N in the microbial biomass was negligible (< 0.001%) at the end of the experiment, and was ≤ 0.007% at all sampling times during the experiment, suggesting that microbial biomass was not a sink for urinary N. There are a number of mass balance studies that have used $^{15}$N to measure the fate of urinary applied N, however, few have measured $^{15}$N recovery in the microbial biomass (Bristow et al., 1987; Wachendorf and Joergensen, 2011). Whitehead and Bristow (1990) reported recoveries of $^{15}$N in the microbial biomass under 700 kg N ha$^{-1}$ urine patch ranging from 2.1-8.1% in the top 50 mm of soil, with a total $^{15}$N recovery of 2.2% at the end of a 321 day study; and Wachendorf and Joergensen (2011) reported recoveries of 7-17% of urinary $^{15}$N in the microbial biomass in the top 150 mm soil over 3 months. Despite the variation in microbial biomass $^{15}$N recovery between these studies, their reported values were all orders of magnitude higher than those measured in the current study.

A study by Bohlen et al. (1999) found that incorporation of $^{15}$N into the microbial biomass is lower where an organic substrate has been applied to the soil surface (in this
case manure), but recoveries of $^{15}$N in the microbial biomass reported here still ranged from 4-10%. Wachendorf et al. (2005) suggested $^{15}$N incorporation into the biomass might be even lower in field conditions where NH$_3$ volatilisation, N$_2$O emissions and NO$_3^-$-N leaching losses occur.

Some field studies (e.g. Ledgard et al. (1988) and Bristow et al. (1987) measured even higher recoveries of $^{15}$N in the microbial biomass after application of $^{15}$N labelled fertiliser. Ledgard et al. (1988) recovered 10-12% of the total soil N in the microbial biomass 3 months after the last $^{15}$N fertiliser application on a Horotiu Sandy Loam. Bristow et al. (1987) recovered 37% of $^{15}$N in the microbial biomass, 2 days following application, with fluctuating recoveries of between 0.1-10% over the next 14 days, which the authors attributed to rapid cycling of N between the soil mineral and microbial biomass fractions. After 370 days, $^{15}$N recovery in the microbial biomass was 0.8% (Bristow et al., 1987), still considerably higher than those reported in this study.

Although these $^{15}$N fertiliser studies give valuable insight into the fate of applied N, previously reported values for the recovery of fertiliser $^{15}$N in the microbial biomass appear to be greater than that for urinary $^{15}$N. Much is still unknown about what drives soil microbial responses to urine deposition (Orwin et al., 2010). Urine deposition introduces a very high N loading, which can result in microbial stress due to NH$_3$ toxicity and a rapid change in osmotic pressure due to the salt content of the urine (Petersen et al., 2004). As such, the application of urine has been observed to reduce microbial biomass and have a large effect on the composition of the affected microbial communities (Wachendorf et al., 2005; Orwin et al., 2010). However, the large amount of N in a urine deposition also stimulates nitrifier and denitrifier activity, and provides a source of water, sulphur and available carbon, which can stimulate microbial biomass growth (Orwin et al., 2010). Not surprisingly, this, along with variability in urine composition (Haynes and Williams, 1993) and antecedent soil conditions, has contributed to the large variability in the reported effects of cattle urine on microbial biomass (Wachendorf and Joergensen, 2011).

The fact that the recovery of $^{15}$N in the microbial biomass was greater in Zone B that Zone A during the first 14 days suggests the urine had an inhibitory effect on the microbial biomass in Zone A. The concentrations of soil NH$_4^-$-N and NO$_3^-$-N were far greater in Zone A than Zone B (Figure 5.25 and Figure 5.27), so presumably NH$_3$ toxicity...
and/or osmotic pressure from the application of urine had a detrimental effect on the microbial population in Zone A, while the microbial population in Zone B assimilated more N by comparison.

Although this may explain the difference in microbial biomass $^{15}$N recovery between Zones A and B, it does not explain the very low recoveries. Some studies suggest that the largest microbial response to additions of N to the soil system occur within the first few hours of urine application (Hodge et al., 2000; Harrison et al., 2008; Inselsbacher et al., 2010). Therefore more insight into the response of the microbial biomass and its assimilation of urinary N could be gained by monitoring changes in the microbial biomass at an hourly scale following N application, rather than the weekly to monthly scale undertaken here.

### 5.5 Summary

- The effective area of a spring deposited urine patch extends beyond the radius of the wetted area by 0.5 m from the outside edge of the wetted area of the deposited urine patch. The urine effect decreased with distance from the wetted area in a non-linear manner.

- There was considerable pasture uptake of urinary N outside the wetted area. Up to 18% of the urinary N was recovered in Zone B and up to 4.2% was recovered in Zone C, resulting in a total of 22% of urinary N being accounted for in the pasture outside the wetted area. This pasture N uptake is thought to be predominantly the result of root proliferation in Zones B and C in response to the high N loading in Zone A. Lateral movement of urine and diffusion of urinary NO$_3$-N could also be responsible for a small proportion of this uptake.

- The effect of fertiliser in combination with urine tended to increase N uptake in both the wetted and effective areas, compared to urine alone but, this difference was not statistically significant.

- Soil N dynamics in the wetted area (i.e. soil NH$_4$+-N and NO$_3$-N concentrations and soil inorganic and organic $^{15}$N recovery) were affected by the application of urine, and while this effect extended to Zone B only, outside the wetted area, the effect was much smaller and short-lived compared to the urine effect on soil N dynamics in Zone
A. The soil microbial biomass outside the wetted area was not significantly affected by urine deposition.

- The effect of fertiliser in combination with urine did not have a significant effect on soil N or microbial N dynamics outside the wetted area of the urine patch.
- Generally, there were no significant interactions between the urine and fertiliser treatments with respect to pasture N uptake, soil N dynamics or urinary $^{15}$N recovery.

These results confirm the hypothesis (a) that a significant proportion of urinary N will be taken up by pasture outside the wetted area of a urine patch. Although plant N uptake tended to increase under concurrent urine and fertiliser treatments, these results were not statistically significant, therefore the hypothesis (b) that concurrently applied fertiliser N (at 35 kg N ha$^{-1}$) and urine (at 800 kg N ha$^{-1}$) will not result in increased pasture growth in the effective area of a urine patch is also accepted. Finally, although the soil N increased for a short time outside the wetted area, the microbial biomass did not, therefore the hypothesis (c) that soil N and microbial biomass N will increase outside the wetted area of a urine patch is rejected.

As the vast majority (> 95%) of $^{15}$N was recovered in Zones A and B, it might be argued that the effective area of a spring applied urine patch in the current study was 0.95 m$^2$, with about $\frac{1}{3}$ of this area representing the wetted area, and the other $\frac{2}{3}$ outside the wetted area. However, given that nearly 5% of the $^{15}$N applied as urine was recovered in the pasture in Zone C, the area of this zone is also arguably part of the effective area, thus making it 2.01 m$^2$. In terms of actual urinary $^{15}$N uptake per m$^2$, the vast majority was taken up within Zone A in the wetted area, with much smaller amounts taken up in the surrounding Zones B and C. Therefore although the total $^{15}$N uptake declined with increasing radial distance from the centre of the urine patch, the area that was affected increased.

The N recovery of up to 22% of urinary N outside the wetted area of the urine patch, and the possibility that the effective area of a urine patch could be up to 6 times the wetted area may have considerable implications at the paddock and farm scale context, not only in terms of our understanding of the total area of the paddock or farm affected by urine patches, but more importantly, in our estimations (predominantly using modelling software) of urinary N uptake by pasture. Caution is required in extrapolating these
results since the response to urine-N is not equal across the effective area. It is important that predictive pasture models are parameterised to account for plant N uptake from the effective area of urine patches for accurate nutrient budgeting and N loss estimates from dairy farming systems.
Chapter 6
Conclusions and recommendations

6.1 General findings

The lysimeter study has provided new information on the fate of fertiliser N following the concurrent application of urine and fertiliser. The results of this lysimeter study were compared with a dynamic process-based model, APSIM, for the purpose of using APSIM as an extrapolative tool to extend the results and information gained from this study beyond what would be practicable experimentally. Finally, a field trial was established to further examine the urinary component of the urine/fertiliser interaction and to determine the effective area of a urine patch.

6.1.1 Lysimeter trial to determine the interaction of concurrently applied urine and fertiliser N on the fate of N

In the lysimeter trial (Chapter 3), $^{15}$N fertiliser was measured in the leachate, from $\text{N}_2\text{O}$ emissions, in pasture and in the soil. The objective was to understand the interaction of N fertiliser and urine timing on the fate of N, where fertiliser is applied following urine deposition, and determine to what extent, if any, N fertiliser enhances N losses from a urine patch.

- **Leaching**

Applied in 8 split dressings per year, the urea fertiliser contributed little to the total NO$_3^-$-N leached ($\leq 2.2\%$). However the results show that when urine is applied with fertiliser, at the 400N rate, cumulative NO$_3^-$-N leaching losses (non-labelled NO$_3^-$-N) increased by up to 55 kg NO$_3^-$-N ha$^{-1}$, compared with urine alone, suggesting greater urine-N leaching, or a greater priming effect at the higher fertiliser rate. The same effect was not observed under the 200N fertiliser rate, in fact cumulative leached NO$_3^-$-N was lower in this treatment, than under urine alone. Furthermore, there was no statistically significant fertiliser rate effect on NO$_3^-$-N leaching.

Urine timing affected the amount of NO$_3^-$-N and DON leached with the greatest losses occurring under autumn applied urine. This was most likely because the autumn urine
application was quickly followed by winter drainage, and climatic conditions were not favourable for optimal plant uptake, therefore NO₃⁻-N losses increased.

- **Nitrous oxide**

Fertiliser ¹⁵N recovery as N₂O-N emissions was very low with recoveries of < 0.05%. This suggests that where urine and fertiliser were applied concurrently, the N₂O emissions were largely urine associated. The treatment induced N₂O emissions as a percentage of the total N applied were also low with emission factors of up to 0.57, 0.42 and 0.35% in the autumn urine, spring urine and nil urine treatments, respectively.

The timing of urine application affected N₂O losses with greater N₂O emissions observed under autumn urine, than spring urine. This is most likely due to favourable conditions for denitrification following autumn urine application, and lower N demand from pasture and soil microbes during this time of year.

- **Pasture N**

Pasture uptake accounted for up to 52% recovery of the applied fertiliser ¹⁵N, however, there was no fertiliser rate effect on the pasture ¹⁵N recovery. Total pasture N uptake was affected by the fertiliser rate with the greatest N uptake measured from the 400N rate, followed by the 200N and 0N rates.

Urine timing also affected pasture N uptake, where the greatest N uptake occurred under spring urine, due to plentiful N availability and optimal environmental conditions for pasture growth following the spring urine application.

- **Soil N**

Recovery of ¹⁵N in the soil fraction at the end of the experiment accounted for up to 22% of the applied fertiliser ¹⁵N with the majority of this in the top 10 cm soil, suggesting that minimal fertiliser associated ¹⁵N was transported beyond the plant root zone.

There was no effect of urine timing on the inorganic, organic or microbial soil N fractions at the end of the experiment. It is suggested that any treatment induced effects on these soil N fractions had disappeared by the time of soil coring (19 months from the beginning of the experiment).
Mass Balance

Total fertiliser $^{15}$N recovery ranged from 68- 81%, leaving 19- 32% unaccounted for. It is suggested the fate of much of this unaccounted for $^{15}$N could have been lost as $N_2$ emissions and/or leached as DON.

Total fertiliser $^{15}$N recovery increased with increasing $^{15}$N fertiliser rate. Fertiliser $^{15}$N recovery was also affected by urine timing with greater $^{15}$N recoveries from spring applied urine than from autumn applied or nil urine. This is likely a result of greater pasture uptake of $^{15}$N in spring due to optimal growing conditions followed by a wet summer, reducing plant water stress. Fertiliser $^{15}$N recovery was greatest from the pasture and soil fractions, and negligible in the leached inorganic N, $N_2O$, roots and stubble fractions, suggesting that the fertiliser N is primarily utilised by the pasture and immobilised in the soil.

Overall there was no significant interaction of the urine and fertiliser treatments on any of the variables measured in the lysimeter trial (Chapter 3) or the field trial (Chapter 5).

6.1.2 Validation of APSIM using experimental lysimeter data

The results of the lysimeter study in Chapter 3 were compared to the results of a simulation in APSIM that mirrored the experimental conditions and management. The objectives were to validate the modelled output data against the experimental data, to determine, based on the relative agreement between the modelled and experimental datasets, whether the APSIM simulation was appropriate for further extrapolative analyses under a greater range of treatments and experimental conditions.

Most modelled outputs were generally within the 95% confidence interval of the measured experimental data. There were some minor incongruities including the fact that the model missed the first drainage event, slightly underestimated NO$_3^-$-N leaching from the autumn urine treatments, and underestimated plant N uptake and growth during the winter months.

The model considerably overestimated $N_2O$ emissions in all the urine affected treatments. This could be due to overestimations of the volumetric soil water content in the model or an underestimation of the saturated hydraulic conductivity. Other potential contributing factors could include the model’s assumption that gas evolved in the soil is emitted immediately, the denitrification coefficient, and the calculation of the $N_2:N_2O$ ratio. As a
result, the APSIM simulation, in its current state, was not considered suitable to immediately perform extrapolative simulations to predict the fate of applied urinary and fertiliser N under a greater range of experimental conditions than that carried out in the lysimeter study (Chapter 3).

The potential parameters responsible for the above incongruities were identified and investigated in an exploratory sensitivity analysis, the results of which are presented in Appendix B.

6.1.3 The effective area of a urine patch

A field trial was undertaken where circular plots were divided into three annular zones, A, B and C. Zone A was designated as the ‘wetted area’ of a urine patch, while Zones A, B and C collectively were the potential ‘effective area’. The key objective of this study was to quantify the extent of the effective area of a urine patch based on pasture response and soil N dynamics.

The effective area of a 0.28 m² spring deposited urine patch extended to a radius of 0.5 m from the edge of the wetted area. The majority (> 95%) of the urinary ¹⁵N recovered was in Zones A and B, suggesting the primary effective area of a urine patch was 0.95 m². However, nearly 5% of the urinary ¹⁵N recovered was in Zone C, presenting a case to also include this as part of the effective area, which would nearly double it to 2.01 m². The urinary ¹⁵N uptake declined with increasing radial distance from the centre of the urine patch.

Approximately 22% of urinary N recovered as ¹⁵N was taken up by pasture outside the wetted area, (with about 30% recovered in the wetted area). This is predominantly due to plant root proliferation in the pasture surrounding the wetted area in response to the high N concentration. Although there was no statistical fertiliser effect, it appeared that the presence of fertiliser in combination with urine tended to increase N uptake in both the wetted and effective areas.

Soil NH₄⁺-N and NO₃⁻-N concentrations and soil inorganic and organic ¹⁵N recovery in the wetted area were affected by the application of urine. This effect extended to Zone B only, but was much smaller and short-lived compared to the urine effect in the wetted area.
There were no significant interactions between the urine and fertiliser treatments with respect to pasture N uptake, soil N dynamics or urinary $^{15}$N recovery. Also, neither urine nor fertiliser in combination with urine had any effect on the soil microbial biomass outside the wetted area of the urine patch.

6.2 Implications

The results of the lysimeter trial (Chapter 3) conflict, to a degree, with the idea that fertiliser applied over top of a urine patch will simply add to the NO$_3$-N leaching load because the N supplied from the urine patch is already surplus to the pasture’s N requirements, and that therefore any additional fertiliser will result in further loss. This was certainly not the case with the applied $^{15}$N fertiliser, as indicated by the low fertiliser $^{15}$N recovery ($\leq 2.2\%$) in the leachate. However, there is evidence that an additional 55 kg NO$_3$-N ha$^{-1}$ of non-labelled N is substituted (or primed) and leached from under the higher (400N) fertiliser rate, but not the lower (200N) rate. Furthermore, the increased plant uptake with increasing fertiliser rate (under concurrently applied urine and fertiliser) suggests that the urine has not saturated the pasture with N as commonly thought. Perhaps the additional fertiliser N uptake by the pasture is luxury uptake, however, it is evidence that a proportion of fertiliser-N applied over a urine patch is utilised by the pasture.

Another implication arising from Chapter 3 was the practicality of the development and investment in precision fertiliser application technology that avoids urine and dung patches. The results suggest that by avoiding fertiliser application over urine patches, leaching losses of fertiliser N will be reduced by up to 2% and N$_2$O emissions reduced by $< 0.1\%$, which seems minimal in terms of total N loss mitigation. However, the apparent substitution of urinary N (or priming of background soil N) resulting in the higher leached NO$_3$-N losses under the 400N fertiliser rate suggests that perhaps the urine patch avoidance technology is more warranted. As described below, more research is required to determine this fertiliser-urine priming interaction and to determine at what fertiliser rates it becomes apparent. These results suggest it would become apparent at a rate somewhere between 200 and 400 kg N ha$^{-1}$ yr$^{-1}$, which is at the high end of the scale of what dairy farm operators in the Waikato region would realistically apply.

The results of the APSIM validation exercise (Chapter 4) have highlighted a key area of the model (the calculation of denitrification and N$_2$O emissions) that requires further
validation and evaluation. The use of APSIM to simulate biophysical processes in New Zealand pastoral systems is increasing, therefore, APSIM’s projection of N₂O emissions needs to be improved in order for it to successfully simulate pastoral N dynamics under urine patches and fertiliser application at a paddock or farm scale.

The results of Chapter 4 also suggest that with some minor improvements to the incongruities mentioned in the general findings above, APSIM generates accurate and robust predictions of drainage, solute leaching, pasture growth and N uptake, thus making it a very powerful tool in the agricultural science field with regards to aiding experimental design, extrapolating field experiments beyond their current cost and labour capabilities and as a potential decision support tool for scientists, farmers, and governmental bodies.

The primary implication of the field trial results (Chapter 5) was that the effective area is not represented by a lysimeter methodology (including that of Chapter 3). Although many studies using lysimeters have not specified if urine was applied over the entire surface area or not, a lysimeter with a diameter of 0.5 m (area of 0.19 m²) is within the range of the average wetted area of a urine patch, (Haynes and Williams, 1993), so presumably any urine treatments would cover this whole area. Because the lysimeter casing creates a barrier to the surrounding soil and pasture, the effective area cannot be accounted for, implying that, the 22% of the pasture urinary N uptake that occurred outside the wetted area in the field (as measured in Chapter 5), may be lost as additional NO₃⁻-N and/or DON leaching and/or N₂O emissions under identical conditions in a lysimeter study. Alternatively, this 22% of urinary N may be taken up by the pasture within the confines of the casing (i.e. the wetted area). Either way, whether there is additional N loss or additional pasture N uptake, the results suggest that in lysimeter studies with no effective area accountability, the measured fate of N may be misrepresented.

This has further implications for the use of models that predict agricultural nutrient dynamics and losses (including APSIM). Many of these models are parameterised and/or validated with data from lysimeter studies (as well as field studies). If the outputs of such models are being increasingly used as justification for farm and/or policy decision making, then it is essential that the data used in their development and evaluation is as accurate as possible.
6.3 Recommendations for further research

Further research is required to better determine the effect of the fertiliser rate on NO₃⁻-N leached under concurrently applied urine and N fertiliser. The two fertiliser rates of 200 and 400 kg N ha⁻¹ yr⁻¹ tested in the lysimeter study (Chapter 3) were insufficient to establish a clear trend of whether fertiliser applied over a urine patch increases the total NO₃⁻-N leached by substituting for additional urinary loss, or priming, or at all. More ¹⁵N studies under the same experimental conditions, which focus on the urinary N component, are therefore needed to better determine this.

Further ¹⁵N studies on the loss of DON from Horotiu soils under concurrently applied urine and fertiliser N are also required to determine the contribution of this fraction to the total ¹⁵N mass balance.

In terms of the APSIM validation study, the next step in future works is to identify the key parameters responsible for the overestimation of N₂O emissions under applied urine, in pastoral systems and carry out a sensitivity analysis to determine which parameters have the greatest impact on the N₂O emissions in the current simulation. These key parameters have already been identified and are listed in Chapter 4. A sensitivity analysis has also been carried out on the identified parameters and is included as Appendix B.

Based on the results of the sensitivity analysis, decisions could be made on which parameters require adjustment, and what their value/s should be adjusted to. It should be noted, however, that model validation against experimental results from a single study are not enough, on their own, to necessitate fundamental parameter changes. Therefore, previous validation studies should be referred to, as well as carrying out further validation against experimental data where urine has been applied in pastoral systems.

The field trial (Chapter 5) findings raise further questions on N leaching and pasture uptake dynamics from lysimeter studies. Where urine is applied to the entire surface area of a lysimeter, are the pasture roots within the lysimeter casing exposed to higher amounts of available N than in the field, resulting in higher yield and N uptake in lysimeters? Or, is the additional N that would have been taken up in the effective area in a field situation, lost via leaching or N₂O emissions in a lysimeter? More research is certainly needed to determine the fate of the 22% of urinary N taken up in the effective area of a field deposited urine patch, under a lysimeter scenario.
Future research should also focus on characterising pasture uptake of urinary N with distance from the wetted area of the urine patch. This study focussed on measuring pasture N uptake from three zones, two of which were outside the wetted area. The results suggest that urinary N uptake by plants declined rapidly with distance from the wetted area (Zone A) to Zone B, however, more information on the nature of this decline with distance is required to better understand the effective area of a urine patch.

Although there was no statistical fertiliser effect on pasture N uptake in Chapter 5, it appeared that the presence of fertiliser in combination with urine tended to increase N uptake in the wetted and effective areas. Further research into this, perhaps with an increased range of fertiliser rates and increased replication, might help determine if this apparent, but statistically insignificant effect, is in fact real. If further research suggests that it is a real effect, it would conflict with the common assumption that fertiliser N, applied in combination with a urine patch, will be lost and not utilised by the pasture due to N loading in excess of what the pasture can utilise in the urine affected area.

### 6.4 Limitations

One of the key implications identified from the field trial in Chapter 5 is the methodology used in lysimeter studies, where urine treatment applications cover the entire surface area of the lysimeters and therefore do not account for urine patch edge effects. This is one of the limitations of the lysimeter study carried out in Chapter 3, as the urine treatments in this study were applied over the entire lysimeter surface area.

One of the limitations associated with both experimental studies is the fact that $^{15}$N labelling can be applied to the urine or fertiliser treatments, but not both where they are applied concurrently. It would have been useful, for example, in the lysimeter experiment (Chapter 3) to determine the proportion of leached NO$_3$-N that was urine-associated, or conversely, in the field trial (Chapter 5) to determine the proportion of pasture N uptake that was fertiliser-associated. If both the urine and fertiliser were enriched in $^{15}$N, it is not possible to differentiate them, so the only way to overcome this would be to duplicate the number of lysimeters and/or field plots and apply $^{15}$N labelled fertiliser to one series and $^{15}$N labelled urine to the other.

The replication of the field plots in Chapter 5 was minimal due to resources and logistics, in terms of the number of plots and treatments (4 different treatments x 4 replicates).
However, the low replication was somewhat redeemed by the zoning system, where within each plot there were three zones, A, B and C. Sampling and analysis was carried out on each individual zone within each plot, effectively trebling the analysis required for each plot, which proved a limitation to having a greater treatment range and/or replication.
References


Anger, M., Hoffmann, C., Kühbauch, W., 2003. Nitrous oxide emissions from artificial urine patches applied to different N-fertilized swards and estimated annual N2O emissions for differently fertilized pastures in an upland location in Germany. Soil Use and Management 19, 104-111.


Ball, P.R., Field, T.R.O., 1982. Responses to nitrogen as affected by pasture characteristics, season and grazing management. Ray Richards Publisher, Auckland.


Bramley, R.G.V., 2009. Lessons from nearly 20 years of Precision Agriculture research, development, and adoption as a guide to its appropriate application. Crop and Pasture Science 60, 197-217.


Harrison, K.A., Bol, R., Bardgett, R.D., 2008. Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biology and Biochemistry 40, 228-237.


Mitchell, K.J., Lucanus, R., 1962. Growth of pasture species under controlled environment. 3. Growth at various levels of constant temperature with 8 and 16 hours of uniform light per day. New Zealand Journal of Agricultural Research 5, 135-144.


Morse, J.L., Bernhardt, E.S., 2013. Using 15N tracers to estimate N2O and N2 emissions from nitrification and denitrification in coastal plain wetlands under contrasting land-uses. Soil Biology and Biochemistry 57, 635-643.


Parsons, A.J., Robson, M.J., 1981. Seasonal changes in the physiology of S24 perennial ryegrass (Lolium perenne L.) 3 Partition of assimilates between root and shoot during the transition from vegetative to reproductive growth. Annals of Botany 48, 733-744.


Stevens, R.J., Laughlin, R.J., 2001. Lowering the detection limit for dinitrogen using the enrichment of nitrous oxide. Soil Biology and Biochemistry 33, 1287-1289.


Weier, K.L., MacRae, I.C., Myers, R.J.K., 1993b. Denitrification in a clay soil under pasture and annual crop: Losses from 15N-labelled nitrate in the subsoil in the field using C2H2 inhibition. Soil Biology and Biochemistry 25, 999-1004.


Appendix A

Introduction to APSIM

A.1 Introduction

Agricultural Production Systems Simulator (APSIM) is a process based model developed by the Agricultural Production Systems Research Unit in Australia (Keating et al., 2003). The purpose for its design was to simulate biophysical processes in farming systems. The APSIM system allows for individual modules or components of a farming system to be plugged in or pulled out by the user (Keating et al., 2003). The modelling framework is broadly made up of simulation modules, defined by the model developer to simulate biological and/or physical farming system processes; data modules, for information input; and management modules, which allow the user to specify rules that are required to characterise and control the simulation (Keating et al., 2003).

APSIM was initially designed to simulate crop systems production and address associated issues. In recent years, the expansion of the range of plant growth modules has seen an expansion in the range of applications of APSIM, including the simulation of pastoral farming systems (Li et al., 2011). The key scientific APSIM modules used in the simulations presented in this study are described briefly below.

A.2 APSIM modules relevant to this study

The information described in this section is summarised from the APSIM Documentation files located on the APSIM website http://www.apsim.info/Wiki/APSIM-Documentation.ashx.

There are a number of essential infrastructure, input and manager modules within APSIM that are not science based. These include the APSIM Clock module where the user is required to specify the beginning and the end of the time period to be simulated. If a start or end date is not specified, the model simulates the entire period of the met file, and if a time-step is not specified, the module defaults to a time-step of 1440 minutes (1 day). The Manager module allows the user to specify a set of rules (using “if” constructs) by issuing messages (many of which are conditional upon events or states) to control the actions of modules within APSIM. Applications of fertiliser to soil, irrigation and
harvesting or sowing crops are all examples of actions that can be specified using the Manager module. Another module, The Operations Schedule is very similar to the Manager module; however this allows the user to specify particular day and year timing of actions for modules in the simulation. The advantage of the Operations Schedule over the standard Manager module is the fixed specification criteria make speed of execution is much faster, as the criteria can be checked and acted upon faster than user-defined logic statements.

The APSIM Met Module provides daily meteorological information to all modules in a simulation. This module requires the information to be in a ‘met’ or ‘weather’ file and the climate parameters need to be specified for each time-step.

The Input Module allows for data, other than model parameters, to be available to all modules. The Input module is an “instantiable” module meaning that APSIM can run zero or more instances of an input module. The Input Module reads two types of data: (a) temporal data (weather data) and (b) data that is constant for any given simulation. The instance name is important. If the Input Module is running as “met”, it will read its data from a single section called “weather”. If not, it will read its data from a single section called “data”.

**A.2.1 Soil N module**

The SoilN module calculates the soil N and C dynamics in each layer. The variables and transformations considered in the module are illustrated in Figure A.1 and Figure A.2 below:

![Figure A.1 Transformations of C and N in each soil layer (Probert et al., 1998).](image-url)
The organic C and N transformations occur between four conceptual pools; fresh organic matter (FOM), biomass (BIOM), humus (HUM) and functionally inert organic matter. The flows between these pools are calculated in terms of carbon. The nitrogen flows are dependent on the C:N ratio of the receiving pool (Probert et al., 1998).

Upon decomposition of any organic matter, carbon dioxide is released to the atmosphere and carbon is transferred to the BIOM and HUM pools. The BIOM pool represents the more labile microbial biomass and microbial products, and internal cycling of carbon occurs in this pool (microbes feeding on microbial products). HUM comprises the less labile, slower decomposing organic matter. Decomposition of fresh organic matter (FOM) is dependent on a C:N ratio factor (Probert et al., 1998). Organic decomposition in each layer is calculated by first order processes that are affected by soil temperature and moisture in that layer.

The processes considered for mineral N transformations include hydrolysis of urea to \( \text{NH}_4\text{-N} \) as affected by soil temperature and moisture; nitrification of \( \text{NH}_4\text{-N} \) using a Michaelis-Menton equation to calculate the potential nitrification rate which is then influenced by soil water, temperature and pH; and denitrification of \( \text{NO}_3\text{-N} \) as affected by a denitrification coefficient, active soil C, soil water and temperature. Mineralisation and immobilisation is calculated as the difference between nitrogen released as a result of decomposition, and nitrogen immobilised as a result of microbial synthesis and humification. Both nitrate and ammonium are available for immobilisation, however ammonium is used preferentially. The reduction of the decomposition rate with depth is accomplished in APSIM by assuming that a certain proportion of organic matter does not decompose (functionally inert). This is achieved by specifying the amount of inert C in each layer at the initialisation stage. The proportion of inert C is calculated and factored into the equation for the decomposition rate (Probert et al., 1998).

**A.2.2 Surface organic matter module**

The SURFACEOM module establishes the overall carbon fraction (0-1); specific area (ha/kg); potential decomposition rate (per day); mineral composition (\( \text{NH}_4 \), \( \text{NO}_3 \) and \( \text{PO}_4 \) (ppm); and the C, N and P fractions in each of the fresh organic matter pools (APSRU, 2012). Decomposition of surface organic material results in the transfer of C and N to the soil system, and loss of CO\(_2\), and is calculated using an exponential decay algorithm.
The factors affecting the decomposition fraction for each component of the surface OM include moisture (moist residues decompose quicker than dry residues); average air temperature; C:N ratio factor; and a soil contact factor, which assumes that surface residues in direct contact with the soil decomposes faster than that accumulated on top, meaning that when large amounts of surface OM is present, over all decomposition rates are lower (APSRU, 2012). The relevant processes in the SURFACEOM module are illustrated in Figure A.2 below. The module outputs can refer to the surface organic material as a whole, or to individual components (e.g. ryegrass) of the surface materials.

The C:N ratio and specific area is calculated separately for each organic matter component, and all organic matter components are included in the calculation of the overall effective cover value (0-1) for the subsequent determination of the effect of surface material on soil evaporation and runoff (APSRU, 2012). Any soluble inorganic N (NO₃-N and NH₄-N) present in the surface organic matter can be transferred into soil pools by leaching in the event of rainfall or irrigation. The default value for the proportion of surface organic matter that is inert from decomposition is zero, i.e. the model assumes that all surface organic material is decomposable. However, the user can specify that a certain proportion of any surface organic is “standing” (inert) using an optional input parameter called “standing_fraction” (APSRU, 2012).

![Figure A.2 Soil organic matter partitioning in APISM and N transformation processes (Meier et al., 2006).](image)
A.2.3 SWIM3

SWIM3 is the most recent release of the SWIM (Soil Water Infiltration and Movement) models. The purpose of SWIM3 in APSIM is to calculate fluxes and storage characteristics of soil water and solutes (Huth et al., 2012). Soil water and solute fluxes are modelled by numerical solutions to the Richards and convection-dispersion equations (Equations 4.1 and 4.2, respectively) (Huth et al., 2012). To do this, the model relies on user input of the basic soil hydraulic properties, including the water content at the drained upper limit (DUL), the lower limit of soil water that plants can extract at 15 bars of pressure (LL15), the soil water content at saturation (SAT), and the saturated hydraulic conductivity (Ks), that is, the rate of drainage at saturation. Adsorption of solute to soil surfaces is calculated by a Freundlich isotherm, where the effects of soil pore space, and tortuosity on solute diffusion are user defined (Verburg et al., 1996b; Huth et al., 2012).

The system and associated flows modelled by SWIM3 are illustrated below in Figure A.3.

As with previous versions of SWIM, SWIM3 operates within a one dimensional soil profile i.e. for a vertical soil profile it will be vertically heterogeneous, but horizontally homogeneous. Consequently, there is only one hydraulic conductivity function for each soil layer, which limits the calculation of any macropore/bypass flow. Also, soil solute...
concentrations will apply to the whole soil layer, so no concentration gradient will exist between the bulk soil and the root zone, which, in reality, affects osmotic potential and water/solute uptake (Verburg et al., 1996b).

SWIM3 also has the ability to automatically adjust its time steps as necessary for rapidly changing processes, whereas calculations carried out by other modules in APSIM generally use a fixed time step of one day (APSRU, 2012).

**A.2.4 AgPasture**

AgPasture is a pasture growth module that enables pasture based systems to be modelled within APSIM, in combination with other land uses (Li et al., 2011). AgPasture is an adaptation of EcoMod, the performance of which has been validated in simulating pasture growth rates in a range of conditions (Cullen et al., 2008; Johnson et al., 2008).

AgPasture integrates a range of pasture species, the functions and processes of which are implemented at the individual species level. This differs from other APSIM plant modules where each species is a separate module. The species compete for resources (radiation, water, N) and their functions and processes include photosynthesis, respiration, biomass accumulation, biomass partitioning across different organs, litter deposition and root senescence (Li et al., 2011). The effects of water and N stress are also accounted for.

AgPasture operates by interacting with other modules in APSIM, for example it takes up N and water from the SoilN module and SWIM and deposits plant litter to the soil surface organic matter (SurfaceOM). Plant moisture demand is calculated using the Penman-Monteith equation. The water available for uptake is calculated by the SWIM module. The soil moisture limiting factor is governed by the ratio of actual plant water uptake to demand. The plant water uptake is equal to the water supply (unless the supply is greater than demand), and the soil water uptake is removed from the soil profile (APSRU, 2012). The soil mineral N supply is in the root zone.

Plant N demand is calculated depending on the maximum and optimum N concentration in new plant tissues, the plant N status, and N remobilisation. The N limiting factor is defined as the ratio of plant available N to plant N demand, and N limitation occurs when the N demand exceeds the N available. The plant available N in the soil is partitioned among species, relative to their individual demands and any plant N uptake is removed from the soil (APSRU, 2012). The effect of water and N limitation on plant growth
results in reduction of new tissue growth, and dilution of N concentration in existing plant tissues (APSRU, 2012). New plant growth is partitioned between roots and shoots by a dynamic partitioning co-efficient which determines a root/shoot ratio. In AgPasture, more new growth is allocated to the shoots in spring (during plant reproductive period), and less in autumn (Parsons and Robson, 1981). Plant tissue turnover exists between four pools: growing, mature, senescing, and dead. The dead plant tissue is transferred to the organic matter pool (SurfaceOM), and senescent roots are transferred to the fresh organic matter pool (FOM) (APSRU, 2012).
Appendix B

Evaluation of a process based simulation model, APSIM: An exploratory sensitivity study

B.1 Introduction

Model evaluation is a fundamental component of environmental model development, and one aspect of model evaluation is sensitivity analysis (Tedeschi, 2006). Sensitivity analysis determines how variations in parameter values within a model affect the model outputs (Jakeman et al., 2006; Bennett et al., 2013). Sensitivity analyses can identify key parameters or inputs in determining model predictions, and the relative sensitivity of the model to these parameters (Shaeffer, 1980; Saltelli and Annoni, 2010). Sensitivity analysis also contributes to building confidence in a model, defining its application limits and identifying areas that require further work (Cichota, 2009).

Sensitivity analysis can also be useful in model verification, that is, ensuring the a model is performing as it was intended (Frey and Patil, 2002; Tedeschi, 2006). For example, if a model generates unrealistic outputs in response to changes in input parameters, efforts can be focussed on these parameters to identify the source of the uncertainty or problem (Frey and Patil, 2002). There are a number of sensitivity analysis methods that can be employed. A review undertaken by Frey and Patil (2002) identifies a number of these, classifying them into mathematical, statistical and graphical methods.

In Chapter 4, the quantitative correspondence between APSIM’s (Agricultural Production Systems Simulator) simulated outputs and the results of a field lysimeter trial were examined. Incongruities between the modelled and experimental data were identified, along with parameters requiring further investigation. The objective of this appendix is to perform an exploratory sensitivity analysis on those parameters identified in Chapter 4 in order to gain a better understanding of which parameters, when adjusted, result in a change in the fate of modelled N under urine deposition and fertiliser application.

The intention of this study was not necessarily to improve shortcomings in APSIM’s current parameter values using only the data generated from the lysimeter experiment (Chapter 3) (although improvements cannot be ruled out). Rather, it was to generate a better understanding of the effects that the APSIM simulation parameter and/or input values had on the simulated fate of N under the same experimental conditions. It is important to note that
the experimental results from the lysimeter study are only a single set of evidence to support or reject scientific theory of N transformations and dynamics in response to urine and/or fertiliser applied to pasture, and therefore this data set may not be reason enough, on its own, to compel fundamental model parameter changes.

**B.2 Methods**

A description of APSIM along with key modules used in the simulations, and model settings, including soil and climate settings was previously described in Chapter 4. The approach and methodology used to obtain the experimental data has been previously summarised in Chapter 4, and described in detail in Chapter 3.

**B.2.1 Exploratory sensitivity analysis: parameters of interest**

This section defines those parameters identified in Chapter 4 that require further investigation into the extent that they affect simulated outputs from APSIM. The degree of uncertainty in how alterations in these parameters, inputs or settings (hereafter all informally referred to as ‘parameters’) affect the modelled outputs, is reflected in changes to the degree of fit between the modelled vs experimental data-sets. The simulation reported in Chapter 4 is hereafter referred to as the ‘base simulation’ and remains identical to that described in Chapter 4. For this sensitivity analysis a ‘one at a time’ (OAT) methodology was chosen so that the effects of each parameter change on individual outputs could be assessed. The parameters were altered over a range of 12 steps with 6 above and 6 below the default or original input value in the base simulation. All other parameter values remained identical to the base simulation.

Although changes in the parameters of interest may affect many output variables, due to the large numbers of parameters, and treatments, a series of key outputs were selected for the sensitivity analysis. These were chosen based on the degree of variation in the modelled and measured data. Outputs where large variation occurred between the modelled and measured data included NO$_3^-$-N leached, drainage, pasture N uptake and N$_2$O emissions. Other outputs included in the sensitivity analysis included cumulative total denitrification, nitrification and denitrification-associated N$_2$O emissions and the N$_2$:N$_2$O ratio; however, these variables were not measured in the lysimeter experiment so were not able to be validated with experimental data. Due to the large number of simulations required, the treatments analysed were reduced to: F0U0, F0UA, F0US, F4UA and F4UA. The rationale behind this selection was to include a control (F0U0), a fertiliser only affected treatment (F4U0), urine only treatments affected by both autumn and spring urine (F0UA and F0US) and the highest N loss risk scenario with fertiliser and autumn urine (F4UA).
Details of the 12 parameters investigated in the sensitivity analysis, including their conceptual role and importance in affecting outputs, experimental evidence and variation in the literature, base setting and model specific information are described in the following sub-sections, with information summarised in Table B.1.

**B.2.1.1 Infiltration depth of urine**

- **Conceptual role and importance in affecting outputs**

  The initial depth of urine movement will be an important factor in the model because this can affect both availability and time that urinary N is available for the pasture to utilise. This, along with climatic factors (e.g. rainfall and evapotranspiration) could potentially result in increased or decreased NO$_3^-$-N leaching. It is thought that the immediate infiltration depth may have contributed to APSIM’s overestimate of NO$_3^-$-N leaching, particularly from the autumn urine treatments, because the urine application was very soon followed by the onset of winter drainage.

- **Experimental evidence and variation**

  Previous measurements of the initial infiltration depth of urine have shown varying results. In a field study by Monaghan *et al.* (1999) where urine patches were applied to pasture, most of the deposited urine (63-73%) remained above 100 mm depth after 6 hours. Similarly, Williams and Haynes (1994) reported that as much as 50% can remain above 50 mm depth. However, conversely, Williams *et al.* (1990b) reported preferential flow of deposited urine reached the bottom of 150 mm soil cores in as little as 11 seconds after application, and Williams *et al.* (1990a) measured that up to 46% of simulated urine (bromide solution) was lost beyond 150 mm depth following application. Snow *et al.* (2011) has also illustrated that increasing the initial urine depth in APSIM resulted in increased NO$_3^-$-N leaching.

- **Base setting and model-specific information**

  In APSIM, the initial depth of urine is a user-input parameter. In the base simulation, urine was applied by adding the equivalent of 800 kg N ha$^{-1}$ urea with the equivalent of 10 mm water (the same N loading rate and volume of urine applied to the lysimeters). Immediately after application (in APSIM), the urinary N concentrations decreased with increasing depth down to a maximum initial depth of 150 mm.
• **Proposed range**

The initial urine depths tested here included 20, 40, 60, 80, 100, 125, **150**, 200, 250, 300, 350, 400, and 500 mm and this was achieved by changing the input value in the APSIM user interface (the parameter value in bold is that used in the base simulation).

**B.2.1.2 Saturated hydraulic conductivity and soil water content**

• **Conceptual role and importance in affecting outputs**

The soil water balance is a critical component of pastoral systems analysis. It is determined by the rates and amounts of water, evapotranspiration and solute flux through the soil system, having direct effects on drainage, NO$_3^-$-N leaching, pasture yields and soil microbial processes such as denitrification and N$_2$O emissions.

• **Base setting and model specific information/experimental evidence and variation**

Soil water and solute fluxes are modelled by SWIM3 within APSIM through numerical solutions to the Richards and convection-dispersion equations (Equations 4.1 and 4.2, respectively) (Huth et al., 2012). To achieve this, the model relies on the basic hydraulic properties, including the water content at the drained upper limit (DUL), the lower limit of soil water that plants can extract (LL), the soil water content at saturation (SAT), and the saturated hydraulic conductivity ($K_s$), that is, the rate of drainage at saturation. The difference between the DUL and LL is the plant available water (PAW). As previously described in Chapter 4, the values of SAT, DUL, LL, and a fourth value, the water content of oven dry soil are used to generate a retention curve across the entire water range (Huth et al., 2012). In SWIM3, SAT, DUL and LL are assumed to correspond to soil matric potentials of -1, -100 and -15000 cm, respectively, however, the matric potential value at DUL can be altered by the user (Huth et al., 2012).

Hydraulic conductivity is calculated by a two-region conductivity function with the user specification of DUL and $K_s$. DUL is point at which drainage becomes a low nominal value ($K_{DUL}$) and $K_s$ is rate of drainage between saturation and DUL (Huth et al., 2012). Hydraulic conductivity is assumed to be 0.1 mm d$^{-1}$ at DUL. Drainage above DUL is calculated by a macropore function (significant *only* above DUL) resulting in the hydraulic conductivity reaching $K_s$ at saturation (Huth et al., 2012).
Figure B.1 An example of how the hydraulic properties of SAT, DUL, LL and oven dry soil are used to generate a continuous retention curve for (a) water content, and (b) hydraulic and saturated conductivity in a silt loam soil (Huth et al., 2012).

In the base simulation, the soil water content tended to remain above the DUL during autumn and winter, for months at a time, then fall below the DUL during summer (with the exception of the high rainfall period in Dec 2011/Jan 2012). The soil water was considerably greater than the DUL at the deepest depth of 200-700 mm. However, the modelled cumulative drainage profile was generally within the 95% confidence interval of the mean experimental drainage profile, thus suggesting the total flux of water passing through the soil is sensible. Although soil water was not measured in the lysimeters over time, the extended periods where soil water was calculated to be above the DUL is questionable. The Horotiu Silt Loam soil is very free draining, and near-saturated conditions do not prevail, therefore it is considered unlikely that the soil water content remained above the DUL for the duration the model suggests.

The position of DUL on the soil water retention curve relative to SAT and LL (in other words, the difference between DUL and SAT and DUL and LL) largely influences the rate of drainage calculated by the model (Figure 4.23a), which appears to be slower than that which occurred in the experiment. This has implications for the volumetric soil water content and the water-filled pore space, and subsequently the denitrification and N2O emissions calculated by the model. The increased water-filled pore space and reduced aeration as a result of the extended high soil water conditions would have been favourable for denitrification. This may potentially explain the greater rates and longer duration of N2O emissions following the autumn and spring urine applications calculated by the model. Furthermore it may also explain why N2 was such a dominant denitrification product in the model, because under prolonged anaerobic conditions, NO3- N is more likely to be completely reduced to N2. The
volumetric water content at DUL (or field capacity) varies temporally and spatially and is dependent on soil physical characteristics such as structure, which governs the volume of soil macropores, and texture, where finer textured soils have a greater water holding capacity than coarse soils. Other factors affecting soil water holding capacity include: soil depth, soil profile layering and the stone content in the soil profile (McLaren and Cameron, 1996).

The position of the DUL on the soil water retention curve also determines the position of $K_{\text{DUL}}$ on the hydraulic conductivity curve (Figure 4.23b) and therefore the slope of the line between $K_{\text{DUL}}$ and $K_S$. The higher the saturated hydraulic conductivity ($K_S$) is relative to $K_{\text{DUL}}$, the steeper the slope of this line, and therefore the faster the rate of drainage between DUL and SAT. Soil saturated hydraulic conductivity ($K_S$) is also affected by structure and texture, where finer textured soils (e.g. clays) have a lower $K_S$ than coarsely textured soils (e.g. sand). Soil profile layering, earthworm activity and plant roots and tortuosity can also affect the $K_S$ of soil (McLaren and Cameron, 1996). Typical values for $K_S$ range from <0.36 mm h\(^{-1}\) in fine textured, poorly drained soils up to >360 mm h\(^{-1}\) in coarse textured soils (McLaren and Cameron, 1996). Reported values of $K_S$ for the Horotiu Silt Loam vary considerably, e.g. Ghani et al. (1996) measured an average $K_S$ of 575 (±176) mm h\(^{-1}\) at 0-75 mm depth, and Singleton and Addison (1999) reported $K_S$ values to range from 64 to 692 mm h\(^{-1}\) in grazed pasture over a range of 50 mm depth increments down to 250 mm.

Another parameter that potentially affects the rate of drainage is the soil matric potential at DUL. The soil matric potential arises as a result of the adhesion and capillary action of water within the soil matrix (McLaren and Cameron, 1996) and is the force that must be overcome by plants in order to take up water from the soil. The lower the matric potential of water in a soil, the greater the force that is required to remove that water from the soil. The matric potential is always negative, because water held by the soil has a lower potential than in a body of free water (McLaren and Cameron, 1996). In the base simulation, the matric potential at SAT and the LL are assumed fixed at values of -1 and -15000 cm, respectively. The DUL has a default value of -100 cm, however this can be adjusted. The most appropriate matric potential for a soil at the DUL may range between -50 to -200 cm (McLaren and Cameron, 1996).

- Proposed range
  
  a) The difference between DUL and SAT was adjusted by calculating proportional % increases or decreases ranging from -60% to +60% of the difference in the base simulation across all depths. The value for SAT was then adjusted to achieve these
differences. In most circumstances if the values of SAT are adjusted in APSIM, the bulk density should be adjusted accordingly. However, due to the fact that this was a simple exploratory sensitivity analysis, with the objective of simply determining the effect of porosity on the selected outputs, it was deemed unnecessary in this instance to alter the soil bulk density characteristics.

b) The difference between DUL and LL was also adjusted by calculating proportional % increases or decreases ranging from -60% to +60% of the difference in the base simulation across all depths. The value for LL was then adjusted to achieve these differences and the value of air dry soil was also adjusted proportionally, so that the water content at air dry soil was always $\frac{1}{3}$ of the LL.

c) The saturated hydraulic conductivity (KS) was adjusted as proportional % decreases and increases ranging from -40% to +40% of the base simulation value across all depths.

d) The matric potential of the soil at DUL was also tested across the range cited in McLaren and Cameron (1996). The values tested included -40, -50, -60, -70, -80, -90, -100, -110, -125, -140, -155, -175, and -200 cm (the parameter value in bold is that used in the base simulation).

### B.2.1.3 Active organic C content of the soil at initialisation

- **Conceptual role and importance in affecting outputs**

  The amount of readily available C in the soil is one of the key limiting factors of denitrification rate and subsequently, N$_2$O emissions. Thus the amount of active organic C in each soil layer at initialisation of the simulation could potentially affect the modelled denitrification and N$_2$O evolution.

- **Experimental evidence and variation**

  Denitrifying bacteria require a readily available C source for the reduction of NO$_3$-N. This is termed ‘active C’ within APSIM ($C_A$). Studies on denitrification and N$_2$O emissions from soil have generally focussed on the topsoil (Murray *et al.*, 2004), as this contains the greatest amounts of organic C and N and the largest microbial populations. Denitrification and N$_2$O production also occurs in the subsoil (Clough *et al.*, 2005), however, it is less well understood, and generally considered to contribute less to the N$_2$O and N$_2$ inventory due to the decline of active soil C and microbial populations with depth.
Some studies have shown that regardless of soil depth, if there is a source of readily available C and/or N there is significant denitrification potential in subsoil (Weier et al., 1993b; Jarvis and Hatch, 1994; Müller et al., 2004; Murray et al., 2004). Clough et al. (1999) incorporated denitrification substrates (NO$_3^-$-N and C) at 0.8 m depth in intact soil cores and observed the greatest N$_2$O production at the 800-1000 mm depth of the cores. In an earlier study, Jarvis and Hatch (1994) demonstrated the occurrence of denitrification in soils at up to > 6 m depth when a source of available C was incorporated; and furthermore, Weier et al. (1993b) observed increases in denitrification at depths of up 1.15 m after the addition of glucose.

Although these studies show the potential for denitrification and N$_2$O production at depth, the likelihood of prolonged availability of a readily available C source (along with NO$_3^-$) at depth is small, therefore it might be expected that N$_2$O emissions resulting from C influxes into the subsoil might be short-lived and sporadic, contributing little to the overall N$_2$O production of a Horotiu soil.

- **Base setting and model-specific information**

The amount of active C affects the denitrification rate of each soil layer in APSIM as illustrated by the equation below (Thorburn et al., 2010):

$$R_{\text{denit},i} = k_{\text{denit}} \times NO_3,i \times C_{A,i} \times F_{\text{moist},i} \times F_{\text{temp},i}$$  \hspace{1cm} \text{B.1}

Where $R_{\text{denit}}$ = denitrification rate at the $i$th soil layer (kg N ha$^{-1}$ d$^{-1}$), $k_{\text{denit}}$ = the denitrification coefficient (= 0.0006), $NO_3$ = the amount of NO$_3^-$-N in the $i$th soil layer (kg N ha$^{-1}$), $C_A$ = the active carbon present in the $i$th soil layer, and $F_{\text{moist}}$ and $F_{\text{temp}}$ = factors (scaled from 0-1) accounting for moisture and temperature limitations on denitrification, respectively, for the $i$th soil layer. The active carbon is calculated using Equation B.2 (Thorburn et al., 2010):

$$C_{A,i} = 0.0031 \times SOC_{\text{ppm},i} + 24.5$$  \hspace{1cm} \text{B.2}

Where $SOC_{\text{ppm},i}$ = soil organic C which is calculated using Equation B.3 from the addition of the C concentrations in the HUM and FOM C pools (Thorburn et al., 2010):

$$SOC_{\text{ppm},i} = HUM_C_{\text{ppm},i} + FOM_C_{\text{ppm},i}$$  \hspace{1cm} \text{B.3}

Where $HUM_C$ = the humus C pool in the $i$th soil layer, and $FOM_C$ = the fresh organic matter C pool.

The inputs to the model for soil C include total organic C (%) (Close et al., 2003), and functions (0-1) for biomass C ($F_{\text{biom}}$) and inert C ($F_{\text{inert}}$) at each horizon in the soil (Cichota et
al., 2013). The active C component is the difference between the total organic C and inert C, after the biomass C has been accounted for; therefore, active C is equal to 1-F\textsubscript{inert} and consists of the humus and fresh organic C pools detailed in Equation B.3.

- Proposed range

The proportion of active C in the soil was altered in APSIM by altering the F\textsubscript{inert} function. The active C (1-F\textsubscript{inert}) was tested at a range of proportional increases and decreases from the base simulation values at each depth. These changes ranged from a 40% decrease to a 40% increase in 10 and 5% increments.

**B.2.1.4 Lower temperature limit for pasture growth**

- Conceptual role and importance in affecting outputs

The growth and N uptake by ryegrass and clover mixed swards is limited by soil temperature, and below certain threshold temperatures, pasture ceases growth. If this lower temperature limit was greater in the APSIM simulation than it was in the lysimeter experiment, then this may have possibly contributed to the underestimation of pasture growth and N uptake calculated by APSIM during the winter months of 2011.

- Experimental evidence and variation

The optimal mean daily temperature for ryegrass dominated swards is 15-20°C (Weihing, 1963) with a minimum of 5°C and a maximum of up to 35°C (Whitehead, 1995). Air temperatures in the Waikato region do not typically reach or exceed 35°C in the summer months, but they do frequently decline below 5°C in the winter months.

Temperature in the topsoil is slightly higher than air temperature, due to the soil’s ability to retain solar heat, but follows similar diurnal fluctuations. Daily temperature fluctuations in the upper subsoil are significantly less due to lagging of heat conduction, and subsoil below 50 cm only follows monthly and seasonal air temperature changes (Singleton, 1991). A study by Li et al. (2011) also observed underestimation of pasture growth using APSIM, when compared to experimental data sets collected during autumn, where air temperatures declined to below average values for this time of year. Li et al. (2011) attributed the model’s underestimation to the likelihood that soil temperature would have been higher than the low air temperatures at this time of year, and that the fact that the model’s temperature restriction on pasture growth is based on air temperature contributed to the underestimation.
Base setting and model-specific information

The temperature parameters in the AgPasture module of APSIM are based on the plant physiological model described by Thornley and Johnson (2000) and as implemented in the EcoMod pasture model (Johnson et al., 2008). The lower temperature limitation on plant growth in the AgPasture module is determined using air temperature (Li et al., 2011). In the base simulation, for perennial ryegrass, *Lolium perenne*, (the dominant grass in the simulation and experiment), the temperature below which pasture growth ceased was 5°C (White et al., 2008).

Proposed range

The lower temperature limit value was tested from 0°C to 8°C in increments of 0.5 or 1.0°C in order to determine the extent that this parameter has on the pasture growth and N uptake over the cooler autumn and winter months. The lower temperature limit in the base simulation may be too high; therefore, smaller temperature increments were tested below 5°C than above it. The values tested include: 0.5, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0°C (the parameter value in bold is that used in the base simulation).

**B.2.1.5 Luxury uptake of N by pasture**

**Conceptual role and importance in affecting outputs**

Under conditions of high N availability, plants may take up ‘luxury N’ in excess of their requirements. This can significantly affect the total N uptake of a fertilised or grazed sward. The N uptake calculated by the model in the harvest immediately following the autumn and spring urine treatment applications was underestimated compared to the measured N uptake. This suggests that the maximum possible N uptake by pasture in APSIM may be less than that which was taken up by the pasture in the experiment.

**Experimental evidence and variation**

On a dry weight basis, plant material usually contains between 1 and 4% N (Hopkins and Huner, 2009). Under conditions of high N availability, plants may also take up ‘luxury N’ in excess of their requirements, where it is stored in the plant tissue as NO\textsubscript{3}\textsuperscript{-} or amides (Whitehead, 1995). Ryegrass dominated pasture, following urine deposition has reported N contents of 3.6-5.2% (During and McNaught, 1961; Lotero et al., 1966; Williams et al., 1989). In the lysimeter experiment, mean pasture N content reached a maximum of 5.1% (F0UA) and 5.5% (F0US) following autumn and spring urine applications, respectively.
• **Base setting and model-specific information**

In the AgPasture module within APSIM, it is assumed that each plant species has a maximum (N\text{max}), minimum (N\text{min}) and critical (N\text{crit}) shoot N concentration during its growth; where N\text{max} is the maximum N concentration that the plants can accumulate, N\text{min} is the N concentration below which the plant will die, and N\text{crit} is the minimum critical N concentration that allows maximum plant growth (Li et al., 2011). Limitation of N on plant growth is defined as the ratio of N uptake to plant N demand, and is termed by a growth limiting factor (GLFn). Plant N uptake can meet or exceed N demand (i.e. luxury uptake), and in this case the GLFn ≥ 1 (Li et al., 2011).

The effect of any N limitations in AgPasture is calculated using Equation B.4 by the construction of an N concentration factor (F\text{Nc}) (APSRU, 2012):

\[
F_{Nc} = \frac{(N - N_{min})}{(N_{opt} - N_{min})}
\]

Where \(N, N_{opt} and N_{min}\) = actual, optimum and minimum N concentration of plant tissue. \(F_{Nc}\) = 1 if \(N \geq N_{opt}\). The maximum N content that can be accumulated in plant tissue in the AgPasture module is 5%; however, above an N content of 4% there is no further N associated growth (Johnson, 2005), therefore, the luxury N uptake has occurred when the pasture N content is between 4 and 5%.

• **Proposed range**

The APSIM simulation underestimated the pasture growth and N uptake in the harvests immediately after the autumn and spring urine applications, suggesting the luxury N uptake component of the base simulation is lower than that which can be accumulated under experimental conditions. The values for the maximum N accumulation in pasture tested here included: 4.0, 4.1, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0 and 6.5% (the parameter value in bold is that used in the base simulation).

**B.2.1.6 Nitrification potential rate**

• **Conceptual role and importance in affecting outputs**

The nitrification rate has a considerable effect on the amount of NO\text{3}^-\text{N} in the soil and can therefore influence NO\text{3}^-\text{N} leaching, denitrification and N\text{2}O emissions and also plant N uptake. The long duration of the modelled soil NH\text{4}^+ peaks following autumn and spring urine application (Figure 4.20) suggest that the modelled nitrification rate was slower than
that which occurred during the experiment and which may have potentially affected that amount of NO$_3$--N available for leaching, plant uptake and denitrification in the model.

- **Experimental evidence and variation**

Nitrification rate depends on soil temperature, moisture and pH, and the supply of NH$_4^+$. It usually starts slowly and increases exponentially until a steady state is reached, before declining when the supply of NH$_4^+$ cannot sustain the increased nitrifier population.

- **Base setting and model-specific information**

In APSIM, ‘potential nitrification’ in the SoilN module is calculated using Michaelis-Menten kinetics. Only the rate-limiting part of the nitrification process, i.e. the conversion of NH$_4^+$ to NO$_2^-$, is represented using a ‘maximum reaction velocity’ (Vmax) and an NH$_4^+$ concentration at $\frac{1}{2}$ Vmax (Km) (Equation B.5) (Meier *et al.*, 2006; APSRU, 2012):

$$ Potential \ rate = \frac{V_{\text{max}} \times NH_4(ppm)}{NH_4(ppm) + Km} $$ \hspace{1cm} \text{(B.5)}

Where *potential rate* is the potential nitrification rate, $V_{\text{max}}$ is the maximum reaction velocity for nitrification (mg N g$^{-1}$ soil d$^{-1}$), $NH_4(ppm)$ is the NH$_4^+$ concentration in soil (mg g$^{-1}$ soil) and $Km$ is the NH$_4^+$ concentration at $\frac{1}{2}$ Vmax (Figure B.2). The daily nitrification is then calculated from the potential nitrification rate accounting for sub-optimal water, temperature and pH conditions.

![Figure B.2 Michaelis-Menten coefficients (Vmax and Km) for a standard APSIM simulation (bold line). The dotted line represents modified coefficients (Meier *et al.*, 2006).](image-url)
The values for Vmax and Km in the base simulation were 40 µg N g⁻¹ soil day⁻¹ and 90 µg NH₄⁺-N g⁻¹ soil, respectively.

- **Proposed range**

Potential nitrification (Vmax) was tested across a range of values above and below the default value to determine the extent of its effect on the modelled outputs. These included 0, 5, 10, 15, 20, 30, 40, 50, 60, 65, 70, 75 and 80 µg N g⁻¹ soil (the parameter value in bold is that used in the base simulation).

**B.2.1.7 Denitrification coefficient (k_{denit})**

- **Conceptual role and importance in affecting outputs**

As well as the effects of active C, soil moisture and soil temperature, the value of the soil denitrification coefficient (k_{denit}) in APSIM may also have a considerable effect on the model’s calculated denitrification and N₂O emissions. It is possible that the default k_{denit} value in the simulation may be too large and thus account for some of APSIM’s overestimation of the measured N₂O emissions.

- **Experimental evidence and variation**

A parameter optimisation conducted by Thorburn et al. (2010) found that APSIM underestimated N₂O emissions under the default parameter values when compared to N₂O emission data from a range of studies including Denmead et al. (2010), Weier et al. (1998), and Robertson and Thorburn, (2007). Although these studies measured N₂O emissions from sugar cane, and bears little resemblance to urine patches and fertiliser effects on pastoral systems, Thorburn et al. (2010) found that changing (increasing) the value of k_{denit} resulted in an improved fit of the modelled data to the measured data from the studies above, suggesting this parameter may have a strong effect on denitrification and N₂O emissions.

- **Base setting and model-specific information**

The denitrification coefficient in APSIM is a multiplier in the model’s calculation of denitrification rate in soil, which is described earlier by Equation B.1 and repeated below:

\[
R_{denit,i} = k_{denit} \times NO_{3,i} \times C_{A,i} \times F_{moist,i} \times F_{temp,i}
\]

The default value for k_{denit} in the base simulation was 0.0006 (Thorburn et al., 2010).
• Proposed range

This was tested at a range of values above and below the default value to determine the extent of its effect on total denitrification and N2O emissions. These included: 0.00006, 0.0001, 0.0002, 0.0003, 0.0004, 0.0005, **0.0006**, 0.0007, 0.0008, 0.0009, 0.001, 0.003, and 0.006 (the parameter value in bold is that used in the base simulation).

**B.2.1.8 Ratio of N2:N2O coefficient (k1)**

• Conceptual role and importance in affecting outputs

The ratio of N2:N2O production affects the total N2O emissions. In the base simulation, the proportion of N2 produced compared to N2O was high, where in all the treatments that received urine, the total N2 emissions over the duration of the experiment were at least 5 times the value of the total N2O emissions. The likelihood of N2 being formed in the model increases under increased WFPS, therefore, the high N2:N2O could potentially be due to the fact that the volumetric water content was above the DUL for extended periods of time (Figure 4.22). The N2:N2O ratio is largely controlled by the ‘k1 coefficient’ which is related to the gas diffusivity in the soil at field capacity which, along with WFPS, predicts the proportion of soil volume that is sufficiently anaerobic to facilitate NO3- reduction to N2O and N2 (Del Grosso et al., 2000).

• Experimental evidence and variation

There is little data on N2 emissions measured directly in the field due because it is notoriously difficult to differentiate N2 emissions from soil from the high background N2 content in the atmosphere. A laboratory study by Monaghan and Barraclough (1993) suggested that losses of N2 from a urine affected soil over a 30 day period were 30-65% of the urine applied, while the contribution of N2O emissions was only 1-5% of the urine applied, which is an even higher N2:N2O ratio than the APSIM simulation estimated. Work by Clough et al. (1996) also postulated that N2 emissions could account for some of the unaccounted for N in 15N balance studies. This suggestion was investigated further in a laboratory study by Clough et al. (2001) who recovered 13.3% of the 15N applied as N2-N, and 9.3% as N2O-N, a much smaller N2:N2O ratio than that predicted by the APSIM simulation.
Nitrous oxide emissions in APSIM are calculated by combining predictions of denitrification (Equation B.6) with a predicted ratio of N\textsubscript{2} to N\textsubscript{2}O evolved (Thorburn et al., 2010). The N\textsubscript{2}:N\textsubscript{2}O ratio is calculated using Equation B.6 (Del Grosso et al., 2000):
\[
\frac{N_2}{N_2O_{denit}} = \text{Max} \left[ 0.16 k_1, \left( k_1 \exp \left( \frac{-0.8 \text{NO}_3_{ppm}}{\text{CO}_2} \right) \right) \right] 
\cdot \text{Max}[0.1, ((1.5\text{WFPS}) - 0.32)]
\]

Where, \( k_1 \) is related to the gas diffusivity in the soil at field capacity (in terms of the proportion of soil that is sufficiently anaerobic for denitrification to occur, and not in terms of gas entrapment in the soil), \text{NO}_3_{ppm} \) is the nitrate concentration (\( \mu \text{g g}^{-1} \) dry soil), \text{CO}_2 \) is the heterotrophic CO\(_2\) respiration (\( \mu \text{g C g}^{-1} \) soil day\(^{-1}\)), and \text{WFPS} \) is the water-filled pore space (\%). The WFPS is affected by the soil hydraulic properties in the model, so the values of these will also affect the predicted N\textsubscript{2}:N\textsubscript{2}O ratio. The value for \( k_1 \) in the base simulation was 25.1.

- **Proposed range**

The value of \( k_1 \) was tested at a range of proportional increases and decreases from the base simulation values at each depth. These changes ranged from a 40% decrease to a 40% increase in 10 and 5% increments and the values were: 15.1, 17.6, 20.1, 21.3, 22.6, 23.8, 25.1, 26.4, 27.6, 28.9, 30.1, 32.6, and 35.1 (the parameter value in bold is that used in the base simulation).

**B.2.1.9 Nitrification coefficient (k\textsubscript{2}): N\textsubscript{2}O production via nitrification**

- **Conceptual role and importance in affecting outputs**

The proportion of N\textsubscript{2}O emissions calculated via nitrification in the model was very small, with the vast majority of N\textsubscript{2}O evolved via denitrification. However, peaks of N\textsubscript{2}O were measured during the experiment that were not associated with rainfall, or conditions conducive to denitrification, therefore it suggests that nitrification played a role in these N\textsubscript{2}O emissions.

- **Experimental evidence and variation**

Nitrification is recognised as a key N\textsubscript{2}O forming process in soils and some studies have shown that production of N\textsubscript{2}O via nitrification in agricultural soils can be substantial under
aerobic soil conditions and when soil NH$_4^+$-N concentrations are high (Bremner and Blackmer, 1978; Pihlatie et al., 2004; Uchida et al., 2012; Morse and Bernhardt, 2013).

- **Base setting and model-specific information**

In APSIM, N$_2$O emissions via nitrification are calculated by multiplying the nitrification rate by a nitrification coefficient (k$_2$). The default value of k$_2$ in the APSIM base simulation was zero; however, normally this value is set at 0.002. This fraction has been reported to range from 0.001 to 0.05 and depending on soil and environmental conditions, is largely dependent on the rate of NO$_3^-$ production (Goodroad and Keeney, 1984).

- **Proposed range**

The k$_2$ coefficient was tested within the range of values mentioned above. These included: 0, 0.0005, 0.001, 0.00125, 0.0015, 0.00175, 0.002, 0.004, 0.006, 0.008, 0.01, 0.025, and 0.05 (the parameter value in bold is that used in the base simulation).
Table B.1  Summary of parameters investigated, default/input values and the range in parameter values tested in the sensitivity analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Base parameter value</th>
<th>Range of tested values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of urine deposition</td>
<td>mm</td>
<td>150</td>
<td>20, 40, 60, 80, 100, 125, 150, 200, 250, 300, 350, 400, 500;</td>
</tr>
<tr>
<td>Difference between DUL and SAT</td>
<td>cm$^3$ cm$^{-3}$</td>
<td>Varies with depth (Table 4.3)</td>
<td>Proportional 60% decrease to 60% increase of default value at 20, 10 or 5% increments, at all soil horizon depths;</td>
</tr>
<tr>
<td>Difference between DUL and LL</td>
<td>cm$^3$ cm$^{-3}$</td>
<td>Varies with depth (Table 4.3)</td>
<td>Proportional 60% decrease to 60% increase of default value at 20, 10 or 5% increments, at all soil horizon depths;</td>
</tr>
<tr>
<td>Saturated hydraulic conductivity (Ks)</td>
<td>mm d$^{-1}$</td>
<td>Varies with depth (Table 4.3)</td>
<td>Proportional 40% decrease to 40% increase of default value at 10 and 5% increments, at all soil horizon depths;</td>
</tr>
<tr>
<td>Soil matric potential at DUL</td>
<td>cm</td>
<td>-100</td>
<td>-40, -50, -60, -70, -80, -90, -100, -110, -125, -140, -155, -175, -200;</td>
</tr>
<tr>
<td>Active C (1-F$_{inert}$)</td>
<td>%</td>
<td>Varies with depth (Table 4.2)</td>
<td>Proportional 40% decrease to 40% increase of default value at 10 and 5% increments, at all soil horizon depths;</td>
</tr>
<tr>
<td>Lower plant temperature limit</td>
<td>°C</td>
<td>5</td>
<td>0.5, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0;</td>
</tr>
<tr>
<td>Maximum luxury N uptake</td>
<td>%</td>
<td>5</td>
<td>4.0, 4.1, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.5;</td>
</tr>
<tr>
<td>Nitrification potential</td>
<td>µg N g$^{-1}$ soil</td>
<td>40</td>
<td>0, 5, 10, 15, 20, 30, 40, 50, 60, 65, 70, 75, 80;</td>
</tr>
<tr>
<td>Denitrification coefficient (k$_{denit}$)</td>
<td></td>
<td>0.0006</td>
<td>0.00006, 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.03, 0.06;</td>
</tr>
<tr>
<td>N$_2$/N$_2$O ratio coefficient (k$_1$)</td>
<td></td>
<td>25.1</td>
<td>15.1, 17.6, 20.1, 21.3, 22.6, 23.8, 25.1, 26.4, 27.6, 28.9, 30.1, 32.6, 35.1;</td>
</tr>
<tr>
<td>Nitrification coefficient (k$_2$)</td>
<td></td>
<td>0.002</td>
<td>0, 0.0005, 0.001, 0.00125, 0.0015, 0.00175, 0.002, 0.004, 0.006, 0.008, 0.01, 0.025, 0.05.</td>
</tr>
</tbody>
</table>
B.2.2 Data analysis

B.2.2.1 RMSE: modelled vs measured data

Where modelled outputs from APSIM were compared with experimental data from the lysimeter trial, the root mean square error (RMSE) was calculated for each variable (Equation B.7). The RMSE expresses the average error in the model-predicted vs mean experimental values in the same units as the variable of interest (Willmott and Matsuura, 2005; Bennett et al., 2013).

\[
RMSE = \sqrt{\frac{1}{n} \sum_{t=1}^{n} (y_t - \hat{y}_t)^2}
\]

Where \(y_t\) is the experimental variable (dependent variable) and \(\hat{y}_t\) is the simulated value for times \(t\); and \(n\) is the number of predictions. A lower RMSE indicated a smaller difference between the modelled and observed values (i.e. a better ‘fit’). The RMSEs calculated in Chapter 4 were compared with the RMSEs of the parameter changes described above for each variable (pasture yield, N uptake, NO\textsubscript{3}\textsuperscript{−} leaching and N\textsubscript{2}O emissions), for the temporal and final value of the cumulative data. The RMSE are reported in table format. Parameter changes resulting in a decline of the RMSE value indicated an improvement in the goodness of fit between the modelled and experimental data.

B.2.2.2 Morris sensitivity analysis method

A sensitivity analysis was performed on all APSIM modelled outputs (i.e. both those that were compared with experimental data, and those that were not). There are a range of methods for performing sensitivity analyses and these have been reviewed previously in Campolongo and Saltelli (1997) and Frey and Patil (2002). The methodology used here is termed the Morris method (Equation B.8) (Morris, 1991), and is a numerical calculation of incremental ratios (called Elementary Effects) for each parameter, from which basic statistics are calculated to provide sensitivity information. The Morris method determines the parameters that have an effect of being either (a) negligible (b) linear and additive or (c) non-linear or involved in interactions with other parameters (Campolongo and Saltelli, 1997). For each parameter, two measures of sensitivity are calculated: (a) the mean, which is a measure of the influence of that parameter on the output, where larger means indicate greater sensitivity and (b) the standard deviation, which estimates the degree of linearity or non-linearity, where a large standard deviation describes a non-linear parameter effect (Campolongo and Saltelli, 1997).
$$E_{y\mid i} = \frac{1}{n_p} \sum_{j=1}^{n_p} \frac{y_j - y_b}{P_{i,j} - P_{i,b}}$$  \hspace{1cm} \text{(B.8)}$$

Where $y$ is the output for a given parameter, $i$; $P$ is the possible value of the output due to changes in the parameter; $j$ is any parameter variation; and $b$ is the value of the base parameter set.

The use of Equation B.8 may be problematic if the parameters being tested have values of different orders of magnitude. For example, one parameter may vary on a scale of hundreds while another varies on a scale of hundredths, making unscaled comparisons a challenge. For this reason, a normalised measure, called elasticity was used (Equation B.9) (Cichota, 2009):

$$E_{y\mid i} = \frac{1}{n_p} \sum_{j=1}^{n_p} \left( \frac{y_j - y_b}{P_{i,j} - P_{i,b}} \frac{P_{i,j} + P_{i,b}}{y_j + y_b} \right)$$  \hspace{1cm} \text{(B.9)}$$

This method of sensitivity analysis is particularly useful when the number of model parameters is high, and/or the model is expensive to compute (Campolongo et al., 2007). Limitations of this method include that any potentially interacting parameters are not accounted for. In order to consider the interaction between parameters (if it exists) the variance method is most commonly used (Ratto et al., 2007; Cichota, 2009), however, the simulations required for this analysis is large (a factorial of the parameters and their variations) making it unfeasible for multiple parameter changes and their effect on multiple outputs in complex models. Other alternative statistical measures include regression or correlation analysis, however, again they are inappropriate for use in models with large numbers of parameters (Cichota, 2009).

**B.3 Results and discussion**

**B.3.1 Measured vs modelled data**

As discussed earlier in Chapter 4, the experimental and modelled results were generally in good agreement, however, discrepancies between modelled and measured data were identified with respect to NO$_3$-N leaching, pasture N uptake, drainage and N$_2$O emissions. The effects of the range of values tested for each parameter on the RMSEs and values of each output are presented graphically. An increasing RMSE with increasing parameter values indicates a decreasing goodness of fit of the modelled data to the measured data, and vice versa. The RMSE and value at the base simulation is always the middle ($7^{th}$) point in the range of parameter values. Unless specified otherwise, all RMSEs and values presented are calculated
based from the final cumulative values at the end of the experiment (both modelled and measured).

**B.3.1.1 Nitrate leached**

Leached NO$_3^-$-N was underestimated by APSIM in the autumn urine treatments (Figure 4.11) Initial urine depth, the nitrification potential and the denitrification coefficient parameters all affected the cumulative leached NO$_3^-$-N in APSIM, while all the other parameters tested had little to no effect.

Initial urine depth had the greatest effect on the cumulative NO$_3^-$-N leached from the autumn urine treatments, which increased with increasing depth of urine at initialisation, decreasing the RMSE (Figure B.5e) and improving the goodness of fit to the measured data. Although an initial urine depth of 400 to 500 mm would bring the modelled cumulative NO$_3^-$-N in the autumn urine treatments to within the 95% confidence interval of the measured data (Figure 4.11), these values are not a realistic representation of initial urine depth in the field (Williams et al., 1990a; Monaghan et al., 1999).

An increased nitrification potential improved the goodness of fit (Figure B.5i) by increasing cumulative NO$_3^-$-N leaching in the autumn urine treatments (Figure B.6i). Decreasing the denitrification coefficient also improved the fit of the modelled data to the measured data (Figure B.4j) by increasing NO$_3^-$-N leached in the autumn urine treatment (Figure B.6j).

**B.3.1.2 Drainage**

Although there was a good fit between the modelled and measured cumulative drainage, the first measured drainage event was not reflected in the APSIM simulation (Figure 4.8 and Figure 4.9). None of the twelve parameter variations had any effect on the day that simulated drainage started (data not shown), and as a result, none of the parameter changes in the sensitivity analysis made any difference to the models delay in capturing the first break through drainage event in the experiment.

**B.3.1.3 N uptake**

There was a generally a good fit between the modelled and measured cumulative pasture N uptake. However, in most treatments, APSIM underestimated pasture growth during the winter months and ‘caught up’ during the spring (Figure 4.6 and Figure 4.7). None of the variation ranges in any of the twelve parameters made any difference to the underestimation of N uptake during the winter months (data not shown). Further investigation into the parameters responsible for these modelled cumulative N uptake and drainage outputs will
therefore be required. It could be argued that in the context of this study, the cumulative results are more important because it was a longer term study of almost 2 years, and the modelled cumulative N uptake and drainage are a good fit to the measured cumulative N uptake and drainage. However, the underestimation of winter pasture N uptake, and lag in the onset of drainage would be problematic in shorter simulations where subsequent pasture growth and/or drainage would not result in the ‘catching up’ observed in the validation study (Chapter 4) and potentially underestimate pasture N uptake and/or drainage.

**B.3.1.4 N2O emissions**

Nitrous oxide emissions were significantly overestimated by the model in all treatments that received urine (Figure 4.13 and Figure 4.14). The parameters that had the largest effect on cumulative N2O emissions were the potential at DUL, the nitrification rate potential, the denitrification rate coefficient, the N2:N2O ratio coefficient and the nitrification coefficient. All other parameters had little to no effect on cumulative N2O emissions.

Decreasing the matric potential at DUL decreased the cumulative N2O emissions in all urine treatments (Figure B.4d iv), bringing the modelled and measured values closer as indicated by the reduced RMSE (Figure B.3d iv). However, even at the lowest variation of -40 cm, the modelled N2O emissions from the autumn urine treatments were still at least double those measured in the lysimeter experiment. Furthermore, a matric potential of -40 cm is not a realistic value for a Horotiu soil at the drained upper limit. Conversely, N2O emissions in all the urine treatments increased with an increasing matric potential (Figure B.4d iv).

Decreasing the nitrification rate potential resulted in decreased N2O emissions (Figure B.8i iv) and a better fit of the modelled to the measured data in all the urine treatments, as indicated by the decreased RMSE’s (Figure B.7i iv). From a nitrification rate potential of 0 to 40 µg N g\(^{-1}\) soil day\(^{-1}\) (default value) there was a steep increase in the cumulative N2O emissions. At parameter values <40 µg N g\(^{-1}\) soil day\(^{-1}\), the increase in N2O emissions plateaued (Figure B.8i iv). A value of between 0 and 5 µg N g\(^{-1}\) soil day\(^{-1}\) (Figure B.8i iv) would reduce the modelled cumulative N2O emissions from the urine treatments, to within the same range as those measured in the lysimeter experiment (Figure 4.14). However, this would result in a considerable decrease of cumulative leached NO\(_3^-\)-N, decreased cumulative drainage and increased cumulative plant N uptake in these same treatments, reducing the goodness of fit for these outputs, thus suggesting such a parameter value is not suitable.

Decreasing the denitrification coefficient also resulted in decreased N2O emissions (Figure B.8j iv) and an increased goodness of fit between the modelled and measured data as
indicated by reduced RMSE values (Figure B.7j iv). Very small changes to the denitrification coefficient resulted in comparatively large differences to the cumulative N\textsubscript{2}O emissions (Figure B.8j iv), for example in the autumn urine treatments, N\textsubscript{2}O emissions climbed from ~5 kg N\textsubscript{2}O-N ha\textsuperscript{-1} at a denitrification coefficient of 0, to 60 kg N\textsubscript{2}O-N ha\textsuperscript{-1} at a denitrification coefficient of 0.001. As mentioned above, a decreased denitrification coefficient would also increase the NO\textsubscript{3}\textsuperscript{-}-N leached in the autumn urine treatments, improving the goodness of fit for this output also. Reducing the value of this parameter appears to be the most appropriate potential parameter adjustment because it reduces the total cumulative N\textsubscript{2}O emissions and increases the NO\textsubscript{3}\textsuperscript{-}-N leached, creating a much better fit between the modelled and measured data for these outputs. A denitrification coefficient value of 0.0001 would bring the modelled N\textsubscript{2}O emissions and NO\textsubscript{3}\textsuperscript{-}-N leaching from the urine treatments to within the 95\% confidence interval of the measured data. However, such a decrease to the denitrification coefficient would also result in small changes to N uptake (Figure B.8j iii) and drainage (Figure B.8j ii), decreasing the goodness of fit between these modelled outputs and the measured data.

An increased N\textsubscript{2}:N\textsubscript{2}O ratio coefficient resulted in decreased cumulative N\textsubscript{2}O emissions, and increased the goodness of fit between the modelled and measured data (Figure B.7j iv). The maximum N\textsubscript{2}:N\textsubscript{2}O ratio of 35 (the default was 25) resulted in a reduction in cumulative N\textsubscript{2}O emissions from the autumn urine treatments from around 41 to 31 kg N\textsubscript{2}O-N ha\textsuperscript{-1} (Figure B.8j iv). This value is still at least three times that of the measured cumulative N\textsubscript{2}O emissions from these treatments (Figure 4.14).

Increases to the nitrification coefficient resulted in increased modelled cumulative N\textsubscript{2}O emissions in the urine treatments, and decreased the goodness of fit with the measured data (Figure B.7l iv). Reducing the nitrification coefficient from its default value of 0.002 did not result in any considerable decrease in the modelled N\textsubscript{2}O emissions (Figure B.8l iv) and did not affect any other outputs. At the smallest value for this parameter, cumulative N\textsubscript{2}O emissions were still at least three times greater than those measured in the lysimeter experiment (Figure 4.14).

None of the parameter variations resulted in any change to the model’s calculation of zero N\textsubscript{2}O emissions from the treatments that did not receive urine. These were all underestimates, as there were small cumulative N\textsubscript{2}O emissions of up to 2 kg N\textsubscript{2}O-N ha\textsuperscript{-1} measured from under the non-urine treatments in the lysimeter experiment. Further investigation may therefore be required to determine how APSIM can account for these background N\textsubscript{2}O emissions under lower levels of fertiliser N.
Figure B.3  Final cumulative RMSEs over the range of values tested in the sensitivity analysis for parameters a, b, c, and d, on 4 outputs: (i) leached NO$_3$-N, (ii) drainage, (iii) pasture N uptake and (iv) N$_2$O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
Figure B.4 Final cumulative values over the range of values tested in the sensitivity analysis for parameters a, b, c, and d on 4 outputs: (i) leached NO$_3$-N, (ii) drainage, (iii) pasture N uptake and (iv) N$_2$O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
Figure B.5  Final cumulative RMSEs over the range of values tested in the sensitivity analysis for parameters e, f, g, and h, on 4 outputs: (i) leached NO$_3$-N, (ii) drainage, (iii) pasture N uptake and (iv) N$_2$O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
Figure B.6  Final cumulative values over the range of values tested in the sensitivity analysis for parameters e, f, g, and h on 4 outputs: (i) leached NO$_3^-$-N, (ii) drainage, (iii) pasture N uptake and (iv) N$_2$O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
Figure B.7  Final cumulative RMSEs over the range of values tested in the sensitivity analysis for parameters i, j, k, and l, on 4 outputs: (i) leached NO$_3$-N, (ii) drainage, (iii) pasture N uptake and (iv) N$_2$O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (µg N g⁻¹ soil day⁻¹)</th>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrification rate potential</td>
<td>(i)</td>
<td>(i)</td>
<td>(i)</td>
<td>(i)</td>
<td>(i)</td>
<td>(i)</td>
<td>(i)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denitrification coefficient</td>
<td>(j)</td>
<td>(j)</td>
<td>(j)</td>
<td>(j)</td>
<td>(j)</td>
<td>(j)</td>
<td>(j)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂:N₂O ratio coefficient</td>
<td>(k)</td>
<td>(k)</td>
<td>(k)</td>
<td>(k)</td>
<td>(k)</td>
<td>(k)</td>
<td>(k)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrification coefficient</td>
<td>(l)</td>
<td>(l)</td>
<td>(l)</td>
<td>(l)</td>
<td>(l)</td>
<td>(l)</td>
<td>(l)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure B.8 Final cumulative values over the range of values tested in the sensitivity analysis for parameters i, j, k, and l on 4 outputs: (i) leached NO₃-N, (ii) drainage, (iii) pasture N uptake and (iv) N₂O emissions; for treatments F0U0, F0UA, F0US, F4U0 and F4UA.
B.3.2 Morris Method results

The sensitivity analysis results using the Morris (1991) method are presented for each parameter on a graph with the absolute mean and standard deviation on the x and y axes, respectively. As previously mentioned, the mean is a measure of the ‘overall’ influence of the parameter in question on the outputs (large means indicate heightened sensitivity), and the standard deviation (x axes) is a measure of the degree of linearity of the parameter effect (large standard deviations indicate non-linear parameter effects) and can also indicate interactions with other inputs. The Morris mean value is the focus here as it is the measure of the overall influence of each parameter on the outputs that we are most concerned with.

The difference between DUL and SAT (Figure B.9a) had the greatest overall effect on leached NO$_3$-N, N$_2$O emissions, total denitrification, denitrification-associated N$_2$O emissions, and N$_2$ emissions with the absolute mean value ranging from 5 up to 32. The control treatment (F0U0) was the most sensitive to variations in this parameter. Although there appears to be no change in, for example, N$_2$O emissions in the F0U0 treatment with changing parameter values (Figure B.4a iv), the actual value changes to the N$_2$O emissions are very small so they are not visible at the scale shown on the graph, yet their change with each parameter variation was large, relative to each other. The standard deviation was also high indicating non-linear effects. Compared to the difference between SAT and DUL, the other 11 parameters had a much smaller effect on the outputs, all with absolute means of < 2.5.

Luxury N uptake had an effect on leached NO$_3$-N, N$_2$O emissions and denitrification-associated N$_2$O emissions, N$_2$ emissions and the N$_2$:N$_2$O ratio, where the Morris mean values were all between 0.5 and 2.5 (Figure B.10h). N$_2$O emissions, total denitrification, N$_2$ emissions, and denitrification-associated N$_2$O emissions were all affected by variations to the nitrification rate potential (Figure B.10i), denitrification coefficient (Figure B.10j), and the N$_2$:N$_2$O ratio coefficient (Figure B.10k), where Morris mean values were all between 0.5 and 1.1. The nitrification coefficient for N$_2$O had the greatest effect on nitrification-associated N$_2$O emissions, but only had a Morris mean value of 1. This parameter also affected total denitrification, N$_2$O emissions and the N$_2$:N$_2$O ratio, with Morris mean values of between 0.5 and 1. The matric potential at DUL affected N$_2$O emissions, total denitrification, N$_2$ emissions and denitrification-associated N$_2$O emissions, with Morris mean values of around 0.9 (Figure B.9d). Active C affected leached NO$_3$-N, N$_2$O emissions, denitrification-associated N$_2$O emissions and N$_2$ emissions, with Morris mean values of around 0.9 (Figure B.9f). All other parameters had effects on outputs where the Morris mean value was ≤ 0.5, and were therefore considered to have had very little effect.
Figure B.9 Morris sensitivity analysis results for parameters a, b, c, d, e, and f, on nine outputs: (i) leached NO$_3^-$, (ii) drainage, (iii) pasture N uptake, (iv) total N$_2$O emissions, (v) total denitrification, (vi) denitrification-N$_2$O emissions, (vii) nitrification-N$_2$O emissions, (viii) N$_2$ emissions, and (ix) the N$_2$:N$_2$O ratio, for treatments F0U0, F0UA, F0US, F4U0 and F4UA. Note the x and y axes are different.
Figure B.10  Morris sensitivity analysis results for parameters g, h, i, j, k and l on nine outputs: (i) leached NO3-, (ii) drainage, (iii) pasture N uptake, (iv) total N2O emissions, (v) total denitrification, (vi) denitrification-N2O emissions, (vii) nitrification-N2O emissions, (viii) N2 emissions, and (ix) the N2:N2O ratio, for treatments F0U0, F0UA, F0US, F4U0 and F4UA. Note the x and y axes are different.
B.4 Conclusions

- Variations to the denitrification rate coefficient appear to be the most promising potential parameter changes as they bring the modelled cumulative N$_2$O emissions and NO$_3^-$-N leaching to within the 95% confidence interval of the measured cumulative values, albeit with small effects to the other outputs.

- Variations (decreases) to the matric potential at DUL and the nitrification rate potential resulted in decreases to the modelled N$_2$O emissions, and an increased goodness of fit to the measured data, however, even at the lowest possible parameter value, the N$_2$O emissions were still considerable greater than those measured during the lysimeter experiment.

- Increases to the initial urine depth would improve the goodness of fit of the modelled and measured leached NO$_3^-$-N data, however, the initial urine infiltration depth required for this improved fit is not a realistic value based on field data.

- No parameter variations had any effect on the day that simulated drainage started or to the underestimation of N uptake during the winter months. Further investigation is required to determine the parameters that affect these.

- The Morris (1991) methodology results suggest that variations to the difference between the DUL and SAT had the largest effect on the modelled outputs with means and standard deviations of up to 33. The Morris (1991) means and standard deviations of all other parameters were < 2.5, with the majority < 1.